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"NORTHERN VIGOUR" POTENTIAL IN MEDICINAL AND AROMATIC PLANTS

Funded by
The Agriculture Development Fund

May 2002

Prepared by: University of Saskatchewan

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"Northern Vigour®" Potential in Medicinal and Aromatic Plants

Funded By:
Agriculture Development Fund (ADF)

May 2002

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ABSTRACT

The current market of medicinal and aromatic crops worldwide is estimated at over $10 billion and is increasing. All seven plants studied in this project are well-established herbs of commerce. The objective of this project was to determine if the Northern Vigor® phenomenon existed in selected medicinal and aromatic crops of considerable commercial interest in Saskatchewan. The phytochemical marker and/or active compounds are known for the plants selected for this study and were used as an index of the potential enhanced concentration under Saskatchewan conditions. The phenological, morphological, and yield differences between Saskatchewan-grown and United States-grown plants were examined in medicinal root crops narrow-leaved purple coneflower *(Echinacea angustifolia),* valerian (*Valeriana officinalis)*, burdock (*Arctium lappa*), dong quai (*Angelica sinensis),* floral crops calendula (*Calendula officinalis),* and aromatic crop catnip (*Nepeta cataria)*. In addition, the effect of seed source on both agronomic and phytochemical yield in milk thistle seeds (*Silybum marianum*) and feverfew aerial parts (*Tanacetum parthenium*) were examined. Seeds were distributed to University of California in Davis and Oregon State University in Madras, and were germinated for transplanting at three Saskatchewan research sites: Outlook, Saskatoon and La Ronge. The significantly higher yield of echinacea and catnip at Saskatchewan locations compared to locations in the U.S. show potential advantage for Saskatchewan. However, differences between locations within Saskatchewan were also significant and the Saskatoon location showed distinct yield advantage for certain crops. Aster yellows is a serious problem for echinacea and valerian crops, however, this disease did not negatively affect root yield of echinacea. Phytochemical studies revealed a strong evidence for the positive latitudinal and environmental effects termed Northern Vigor® on phytomedicinal quality of root crops particularly echinacea, and to some extent valerian and burdock. Saskatchewan does not seem to have a competitive advantage over Madras, Oregon, a prime herb production area in Pacific Northwest, in growing catnip and calendula. Good quality feverfew and milk thistle can be grown in Saskatchewan, but further studies into optimizing seeding date and improving uniformity of seed maturation is required for milk thistle.

EXECUTIVE SUMMARY

The market value of medicinal and aromatic crops worldwide is estimated at over $10 billion. The retail value of the US phytomedicinal market was established at US$1.5 billion in 1995. The worldwide increase in demand for plant-derived drugs and natural compounds for a variety of applications has increased interest in medicinal and aromatic plants as alternative cash crops across the Canadian prairies. The objective of this project was to determine if the Northern Vigor® phenomenon, expressed as a combination of elevated crop yield and phytomedicinal quality, exist in seven medicinal and aromatic crops that are well-established herbs of commerce worldwide. Specifically, it was of interest to determine whether Saskatchewan climate is conducive to production of superior quality botanicals in comparison to more southern locations such as Madras, Oregon, and Davis, California. Section one of this project which addressed agronomic performance, primarily crops yield, was supervised by Karen Tanino, and section two which addressed crops phytomedicinal quality was supervised by Branka Barl, Department of Plant Sciences. This project is an extension of a one-year preliminary study conducted conducted in 1997 (ADF project # 96000172).

The following crops were studied for the phenological, morphological, and yield differences, and phytochemical differences between Saskatchewan and United States sites: medicinal root crops narrow-leaved purple coneflower *(Echinacea angustifolia)*, valerian (*Valeriana officinalis)*, burdock (*Arctium*

lappa) and dong quai (*Angelica polymorpha sinensis),* floral crops calendula (*Calendula officinalis,)* and essential oil bearing crop catnip (*Nepeta cataria)*. In addition, the effect of seed source on both agronomic performance and phytochemical quality of milk thistle seeds (*Silybum marianum*) and feverfew aerial parts (*Tanacetum parthenium*) were examined. Seeds were distributed to University of California in Davis and Oregon State University, Madras campus. Saskatchewan sites consisted of La Ronge, Saskatoon and Outlook. All crops were harvested at the same developmental stage and shipped to the University of Saskatchewan for phytochemical analysis.

From an agronomic perspective two crops showed potential advantage for Saskatchewan, echinacea and catnip. Location effect was significant for most crops, even among sites within Saskatchewan. Aster yellows significantly affected both echinacea and valerian crops, however, this disease did not negatively compromise the yield of echinacea. Significant yield differences based on seed source were also determined for milk thistle. Fall seeding should be examined for echinacea, burdock and milk thistle crops. This seeding practice should also be tested in catnip and valerian to reduce costs.

Echinacea showed good stand establishment except in Davis both in 1999 and 2000. Site x year interaction was significant. In 1999, both Saskatoon and Outlook sites produced significantly greater root fresh weight of the one-year crop compared to La Ronge and Oregon locations. In 2000, Saskatoon, Outlook and Oregon all produced similar fresh weight yields, which were greater than La Ronge. The Saskatoon location again consistently produced the highest first year root yields of echinacea. Aster yellows affected the crop but a comparison of root yield from infected and non-infected crops did not show significant root biomass yield reduction due to this disease. The catnip crop consistently produced higher dry weight at the Saskatoon site over both years of testing. The Oregon site also produced significantly high dry matter in the year 2000, which was equivalent to the Saskatoon location. Oregon is currently the largest producer of mint in North America. The level of response was year-dependent and ranged from 48% to 300% greater dry weight of Saskatoon-grown plants over Davis, California. Flowering date was earliest in Oregon and latest in La Ronge. Good stand establishment (100%) after transplanting was evident at all locations but fall seeding should be assessed.

The valerian crop was severely infected with aster yellows, which, unlike the echinacea crop, significantly reduced harvestable root yield. Crops expressing advanced stages of this disease had 9-fold lower yield compared to early stage infected crops. Notably, a distinct location x condition response was observed where plants infected with the same level of aster yellows produced greater root biomass in Saskatoon compared to La Ronge and Outlook. Burdock was a vigorous plant when transplanted but did not germinate when seeded in the spring. However, numerous volunteer seedlings from the previous year's growth were evident and thus, fall seeding might be a viable alternative to spring transplanting. Oregon had extremely low dry weight yields on the one-year-old crop. Saskatchewan-grown burdock had equivalent dry weight yields to that grown in California. While burdock shows potential, this crop is extremely difficult to harvest and roots are found 3 feet below ground. If a mechanical harvester can be developed, burdock may be a promising crop for the northern Saskatchewan regions.

Calendula crops had good stand establishment at all sites and in both years. Planting dates were similar but date of first harvest of the flower heads varied among all sites except in Davis, CA, where it consistently occurred on July 17th. Location, date of harvest and the interaction effect was significant in

1999. Only the interaction was significant in 2000. Mean yield was variable for Saskatchewan sites between both years. Oregon had the highest mean per week yield followed by La Ronge, Davis, CA, Saskatoon and Outlook in 1999. Whereas in 2000, Davis, CA, had the highest mean dry weight followed by Saskatoon and Outlook.

Both seed source and harvest date were significant in the milk thistle crop. Of the three seed sources: Bulgaria, Oregon and Maine (USA), the Bulgarian seed produced the highest seed yield with over 200% greater seed yield compared to Oregon and Maine seed sources. All seed heads were collected at the same stage of development over 5 weeks of sampling. September 26th represented the date with the highest harvestable seed yield. Fall seeding is a viable option for this crop which, when compounded with projected global climate change extension to the Saskatchewan growing season renders milk thistle a crop of promise for this province. Feverfew data shows potential biomass differences between sources from Richter's (Ontario), Horizon Herbs (Oregon) and Johnny's Selected Seeds (Maine), however, definitive conclusions cannot be made without further testing. Nevertheless, with the same planting date, Johnny's Selected Seeds appeared to have similar upper plant biomass but greater floral dry weight compared to Horizon Herbs. All sites had serious establishment problems with *Angelica sinensis*. In Saskatchewan, the disease infecting this crop was identified as *Colletotrichum* species.

Phytochemical studies revealed a strong evidence for the positive latitudinal and environmental effects termed Northern Vigor® on phytomedicinal quality of echinacea. Data suggested that Saskatchewan has a competitive advantage in producing premium quality echinacea root in North America. Levels of two classes of natural compounds used by the industry as quality indicators were consistently higher in Saskatchewan-grown root. In both 1999 and 2000, the average content of echinacoside, a dominant phenolic compound, in one year echinacea root was twice as high across Saskatchewan sites (1.36%) in comparison to Oregon site. Relative content of alkylamides was on average 61% higher at Saskatchewan sites than Oregon site. Two year root contained lower amount of both echinacoside and akylamides than a one year old root from the same Saskatchewan site (e.g. 0.97% vs. 1.40% in Saskatoon and 0.89% vs. 1.05% in Outlook). With respect to the economics of echinacea root production, further investigation into the optimal production cycle (one year, two-year and three year) with respect to both yield and quality is required. Aster yellows disease, while significantly affected yield, did not seem to have an obvious negative effect on quality.

The valerian root from Saskatchewan had significantly higher essential oil content (0.27%) than valerian root from US sites (0.19% in Madras, 0.15% in Davis), and was on the lower end of the expected range, 0.3 to 0.7%. The essential oil content in a similar study conducted in 1997 (ADF Project #96000172), was the highest in the Saskatchewan-grown root and was on the high end of the range, 0.78%. The content of valerenic acids was similar in Oregon- and Saskatchewan-grown roots (0.23 to 0.26%), but significantly higher than in California-grown root. The highest content of valerenic acid in a single root grown in Saskatchewan was 0.42% (La Ronge). Aster yellows disease, regardless of the stage of disease development, did not seem to impact valerenic acids content. Considering that phytomedicinal quality of valerian is determined by the content of both valerenic acids and essential oil, the results of this study suggest that Saskatchewan has a competitive advantage in valerian production over Madras, a prime area for herb production in Oregon. However, given severe impact of aster yellows disease on root yield and crop survival, it is imperative that advances be made in the management of this disease, before true potential of valerian in Saskatchewan can be properly assessed.

Burdock root grown in Saskatchewan contained on average 0.73% chlorogenic acid, which is 228% the content of Davis-grown root. The content of three other unidentified compounds, likely phenolic acids related to chlorogenic acid, was also proportionally higher in roots from Saskatchewan. These data suggest a strong possibility of a Northern Vigor® response with respect to phytomedicinal quality of burdock root.

Phytomedicinal quality of calendula flowers was determined by the flavonoid content, specifically by the levels of three flavonol glycosides: isorhamnetin-3-O-2-rhamnosyl-rutinoside, isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside. Over two years of study, flowers grown in Madras contained up to 35% more flavonoids than flowers grown in Saskatchewan. The total flavonol glycosides content ranged from 0.8% in Davis to 1.43% in Madras. Flowers from all three Saskatchewan sites were of comparable quality with average flavonoid levels of 1.0 to 1.1%. Amount of flavonoids in flower petals decreased progressively from July to September. While Saskatchewan does not appear to have a competitive advantage over Madras in calendula production, it has been proven that good quality calendula can be grown as far north as La Ronge, providing recommended harvest and post-harvest handling procedures are followed.

Catnip was analyzed for the content of essential oil, which varied greatly from year to year and between locations. Catnip flowers are the richest source of essential oil yielding 0.69%-0.84% of oil, followed by leaves, 16%-0.18%. Main constituent is nepetalactone accounting for 93.8%-95.6% of oil in flowers and 72-76% in leaves. Stems contained no essential oil. Essential oil yield and composition was tested using pilot scale steam distillation unit at CDC-South in Brooks, Alberta. Comparable results were obtained for both pilot scale and laboratory scale. The two year study aimed at probing latitudinal and environmental effect on quantity and quality of essential oil from catnip provided no encouraging results. It does not appear that Saskatchewan has any competitive advantage over Oregon in growing catnip. Our data indicated however that seasonal influences, harvest time and practice, particularly with regard to the height of the cut and post-harvest handling are of great importance for commercial production of catnip for essential oil.

Feverfew of good quality can be grown as an annual plant in Saskatchewan, but Saskatchewan does not appear to have any competitive advantage over other locations in growing this medicinal plant. Parthenolide content was in the range from 0.06% to 1.16 % and was the lowest in pre-flowering leaves followed by leaves, top half of the plant and flowers. Parthenolide content was more than 50% lower in top half of a plant than in flowers alone. Parthenolide content was also influenced by seed source. Milk thistle seed of acceptable quality could be grown in Saskatchewan based on the content of active compounds known as silymarin complex. Silymarin content was lower in early September harvests (up to 1.8%) than in later harvests of October 4 and 12 (up to 2.4%) and was greatly impacted by seed maturity. Data suggested that milk thistle requires 115-120 days from transplanting to harvest, but that seed can be harvested after a hard frost without compromising crop quality. This aspect of flexible harvest date is a critical factor in the success of milk thistle as a medicinal crop for Saskatchewan. Fixed oil content ranged from 22.8 to 26.1% between seed sources. Seed from Bulgaria and Richters resulted in crops with significantly higher amount of both silymarin and fixed oil, suggesting the importance of seed source. Overall, milk thistle has some potential as a medicinal seed crop for Saskatchewan, but further research into optimizing seeding date and improving uniformity of seed maturation is required for optimizing seed quality and consistency.

Section A) AGRONOMIC STUDIES

1.0 Introduction

Vegetatively propagated crops produced in Saskatchewan are significantly more productive than plant material grown in more southern latitudes in the U.S.A. This phenomenon has been termed Northern Vigour ® and has opened a major export market for Saskatchewan-grown seed potatoes. In addition, significant yield increases from plantings of Saskatchewan-grown strawberry crowns (40 – 60% in the first three months) and garlic (40% yield increase and 200% elevation of essential oils) over California-grown plants have been recently demonstrated by our group on another ADF project. Collectively, it indicates a unique plant response to Saskatchewan's climate with a significant competitive advantage over other regions.

2.0 Materials and Methods

The project consisted of five sites in total, three within Saskatchewan (Outlook, La Ronge and Saskatoon) and two in the United States (Davis, CA; West Lafayette, IN; Madras OR. The Indiana site was subsequently dropped due to continuing problems at that site. From each location the phytochemical, phenology and yield differences between Saskatchewan-grown and United States-grown plants were studied in: valerian (*Valerian officinalis)*, calendula (*Calendula officinalis)*, catnip (*Nepeta cataria)*, burdock (*Arctium lappa)*, and narrow-leaved purple coneflower (*Echinacea angustifolia)*. In addition, the effect of seed source on both yield and quality in milkthistle (*Silybum marianum*) and feverfew (*Tanacetum parthenium*) were examined. Milkthistle sources were Bulgaria, Horizon's Herbs from Oregon and Johnny's Selected Seeds from Maine. Feverfew was sourced from Horizon's Herbs and Richter's Seeds (Goodwood, Ontario).

Seeds of Calendula were ordered from Richter's Herbs in Goodwood, Ontario and catnip, valerian, burdock and echinacea seeds were received from Elixir Botanicals in MO. USA. Dong Quai seeds were ordered from Horizon Herbs in Williams, OR. Seeds were couriered in March of each year to the U.S. cooperators – Herb Phillips at the University of California at Davis, CA; Dr. Jim Simon at Purdue University in West Lafayette, IN; and Dr. Peter Sexton of Oregon State University, research station at Madras, OR. Each site germinated and grew seedlings needed to complete the plot requirements of 120 plants. Plants for all Saskatchewan sites were propagated in the Agriculture Greenhouses at the University of Saskatchewan.

Although soil moisture, type, fertility, etc. are factors that may influence levels of active compounds and essential oils, it is impossible to hold all of these constant between sites. Thus, the following was suggested and the guidelines were distributed to all cooperators:

1. Irrigation: Irrigate as local conditions warrant to maintain adequate soil moisture levels.
2. Fertility: Based upon soil analysis, bring total nitrogen up to 80 lbs./acre and total phosphorus up to 80 lbs./acre. Either 16-20-0 or 23-23-0 are good nitrogen and phosphorus fertilizers that could be used to broadcast and incorporate prior to

planting. For nitrogen application, a split application is used with one third applied at planting and two thirds applied one month later. Please include your soil tests in the results.

3. Weather data: Please include local weather data (temperature (maximum and minimum and precipitation)
4. Crops: Calendula (*Calendula officinalis*), Catnip (*Nepeta cataria*), Burdock (*Arctium lappa*), Chinese Angelica or Dong Quai (*Angelica polymorpha sinensis*), Valerian (*Valeriana officinalis*), Echinacea (*Echinacea angustifolia*).
5. Plot plan: Please arrange plots to fit your existing irrigation and space availability. The reps are arranged together to minimize potential shading and allelopathic effects between different crops. A suggested plot plan will be sent with the seeds but can be modified according to your local field plan.
6. Reps: Four reps and two guard rows.
7. Plants/rep: 20
8. Number of harvested plants: middle 10 plants/rep/crop
9. Between row spacing: Again, this should fit into your existing equipment but we recommend roughly 4 feet.
10. Interplant spacing: Crop-dependent and instructions will be mailed with the seeds (for rough estimate: see below).
11. Harvest: Again, crop-dependent and instructions will be mailed.
12. Data to be collected by each location:

- Planting date
- Stand establishment
- Date of first flowering (where applicable)
- Date of harvest
- Harvested yield (fresh and dry weight)

The protocol for germination was provided with the seeds. The basic protocol involved seeding trays using a seed mix (e.g. Sunshine mix #5), then transplanting into plug trays (Sunshine mix #4) before placing out into the field. The number of plants needed per crop was 120 plants (4 reps and 2 guard rows, 20 plants per rep). The most uniform 120 plants were selected for transplanting. Since there were 6 crops, this translated into 720 plants in total (See Calendula and Catnip example below). Note: After transplanting into plug trays, cooperators were asked to irrigate as necessary and fertilize once per week with 20-20-20 (concentration of 250 ppm). Since the planting season was unpredictable based on the weather, we suggested 2-3 staggered plug plantings so the best transplants may be selected. All material was requested to be stored at 10°C after drying until shipping.

Each plot (4 reps and 2 guard rows) varied slightly in size due to differences in planting density but an outside estimate was 20 feet wide by 40 feet long. Thus, each plot was roughly 800 square feet. With 6 plots (crops), this represented a total field area of 6 x 800 = 4800 sq. ft. This did not include the 4-foot space between the crops

Table 2.1 Agronomic information provided to all cooperators.

Crop	Germination	Within Row Spacing	Harvestable Yield
Catnip	Place seed on moist filter paper in petri-dishes. Then place the dishes in room temperature (20-25°C) with plenty of day light. Approx. 10 days to germination with 60% emergence. Keep filter paper moist. As seeds germinate transplant them into plug trays in the greenhouse (approx.24°C).	18" (45cm)	When plant material has >80% of all spikes in full bloom, cut the plant at the base stem removing the entire plant except the roots. Place each plant in a separate large brown paper bag leaving the top open (You may have to bend the plant in the bag to make it fit!). Have each bag labeled with Rep #, Plant # and Date of Harvest. You should then have ten bags per row x four rows equals 40 bags of material. Take the fresh weights of each bag and record it on the bag and in a data sheet. Place bags in a 30°C dryer to remove excess moisture. After two – three days the material should be adequately dry. Dry is determined when no further reduction in weight due to evaporative water loss occurs. Take dry weights and record them on in the same data table and on the bag again. Fold over the tops of the bags and staple shut. Randomly choose three of the bags per replicate (therefore a total of 12 bags will be sent) and ship them with the data sheets to us. If necessary store at 10°C until shipping. The rest of the material may be discarded.
Calendula	Can either direct seed into plug trays, or germinate in petri dishes with moist filter paper in the greenhouse under daylight conditions. 40% germination in approx. 10 days	18" (45cm)	When around 75% of the plant material is flowering, the first harvest should be initiated. Collect all flowers that have not started to wilt or form seedpods, please remove these and discard them. This will allow plant energies to be put into continuous bloom. Remove the flower heads with as little stem as possible attached. Place each row in a separate paper bag marked with replicate number, and date of harvest. Take fresh weights of each and mark on a bag and on a data sheet. Place bags in a 30°C dryer or lay out material to dry on screens away from the sun. When dry, (dry is determined when no further reduction in weight due to evaporative water loss occurs) record the dry weights for each rep. Place back in paper bags if drying was done on a screen. Place in 10°C storage until shipping. Please do this on a weekly basis, until calendula yields noticeably decline. Do not bulk the samples – keep them in their separate bags which they were dried in.
Angelica	This is a cold soil germinator 42F or 5°C. Press seeds into a tray of Sunshine Mix #5. Keep in the light	24" (60cm)	This is normally harvested in the second season, but we will take a small sub-sample of four plants per rep this year for analysis. Dig roots before freeze up. Place individual plants in separate bags with the replicate number and plant number on them. Wash roots thoroughly after removing the upper portion

	and maintain moist soil. You will notice the seed imbibing water and germination will be slow under these cold temperatures. **It may take a month for germination.** Remove seedlings and transplant to plug trays and place in 24°C greenhouse.		(try and trim it as close to the root as you can leaving only a small section). Take fresh weights and place bags in the dryer at 30°C. When dry, (dry is determined when no further reduction in weight due to evaporative water loss occurs) take dry weights and write them on the corresponding bags and data sheet. Place in 10°C storage until shipping. Send all samples.
Burdock	Place seed in petri dishes with moist filter paper in the greenhouse at 24°C with daylight, or direct seed into plug trays. Unsure as to the number of days to emergence, therefore stagger plantings to ensure proper plant sizes for transplanting in the field. Germination rate is 70-80%.	24" (60cm)	This is usually harvested in the second year – but we will take a small sub-sample this year for analysis. Take four root samples from the first five plants at the front or back of the row. Sample should consist of four plants per replicate. Collect the roots on Nov.1. Trim top off plant and wash roots thoroughly. Ensure that only a very small portion of the stem is left. Take fresh weights of roots from each individual plant and place them in a paper bag. On the bag, please mark the replicate number, the plant number, and the fresh weight. Place the bags in a 30°C dryer. When dry (dry is determined when no further reduction in weight due to evaporative water loss occurs) take dry weights, record these on the bags and place in 10°C storage until shipping. Please mark all measurements on a data sheet as well.
Echinacea	Take seed/vermiculite mixture and spread thinly on a number of seed trays. Cover lightly with Sunshine Mix #5 and place in greenhouse. Water regularly. When sprouting occurs one can then transplant into plug trays.	18" (60cm)	As this will be the first year for the new plants – we will harvest a small sub sample of material for analysis, four plants per row (see burdock above). Please dig roots on Nov.1. Trim stem down to the root portion – leave a little bit (1-2cm) of stem attached. Clean roots thoroughly with water. Place each root with replicate number and plant # on the bag. Take fresh weights and mark them on the bag and the data sheet. Place roots in 30°C dryer. Once dry, take dry (dry is determined when no further reduction in weight due to evaporative water loss occurs) weight measures and write on bag and data sheet. Store in 10°C cooler until shipping. For those you have viable plants from the 1999 planting – please take a 4 plant/rep subsample. We will harvest everything in 2001.
Valerian	Germinate on moist filter paper in petri dishes in the dark at	(40cm)	1st and 2nd year growth will be sampled. Please dig the roots on Nov.1. Trim off the tops of the valerian so that only a very short stem is left (1-2cm). Place

	room temperature. Note: inhibitors in seed coat so rinse filter paper regularly. Once germinated, transplant into plug trays in the greenhouse. Previously: 60% germination in 10 days.		roots in individually marked bags with the replicate and plant number on them. Wash the roots thoroughly (I find a spray nozzle on "jet stream" works the best for this). All soil must be removed. Let the roots air-dry slightly (a couple of minutes) until the water from the wash has dried off. Then take fresh weights. Please mark the fresh weights on the paper bags. Place the bags into the dryer at 30°C. Once roots are dry, (dry is determined when no further reduction in weight due to evaporative water loss occurs) collect the dry weights – mark them on the bags and place in 10°C storage until shipping. Please mark all data on data sheets as well.

Feverfew: Germination under 25°C greenhouse. Seedlings were fertilized weekly with a five hundred (500g/L) solution of 20:20:20 (N:P:K). Seedlings were hardened off under a shade house and then planted at the Horticulture Field Lab. Soil moisture levels were monitored and watered as necessary.

Milkthistle: Germination of the seed was initiated over a duration of four weeks starting the last week of April, 2001 within a 25°C greenhouse. Seedlings were fertilized weekly with a five hundred (500g/L) solution of 20:20:20 (N:P:K). Seedlings were placed outside to harden off during the last week of May and planted out on the 5th of June 2001. Fields were fertilized prior to planting by bringing soil levels of N, P, and K up to 80lbs/acre based on soil analysis performed by Enviro-Test Agricultural Services. Seedlings were planted at a 1m inter-row spacing and at a 2.75m between row spacing. Soil moisture levels were monitored and watered as necessary.

Table 2.2 Example Data sheet:

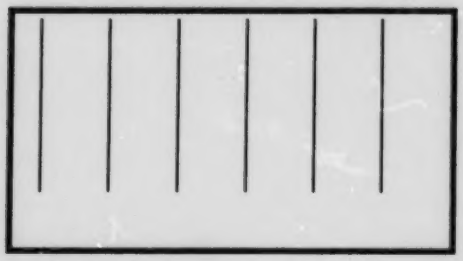
Site Location:
Crop:
Date of Planting:
Date of First Flower:
Date of Harvest:

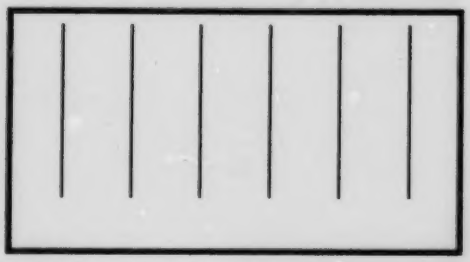
Replicate	% Stand Survival	Plant #	Plant Height at Harvest (cm)	Fresh Wt. (g)	Dry Wt. (g)	Comments
		1				
		2				
		3				
		4				
		5				
		6				
		7				
		8				
		9				

		10			
		1			
		2			
		3			
		4			
		5			
		6			
		7			
		8			
		9			
		10			

Calendula

Catnip





Note: the above plots kept the 4 reps and 2 outer guard rows together within the same crop but avoided shading and any allelopathic effects.. The crops were arranged to fit each cooperator's field plan. The other crops are not listed but were suggested to be planted similarly.

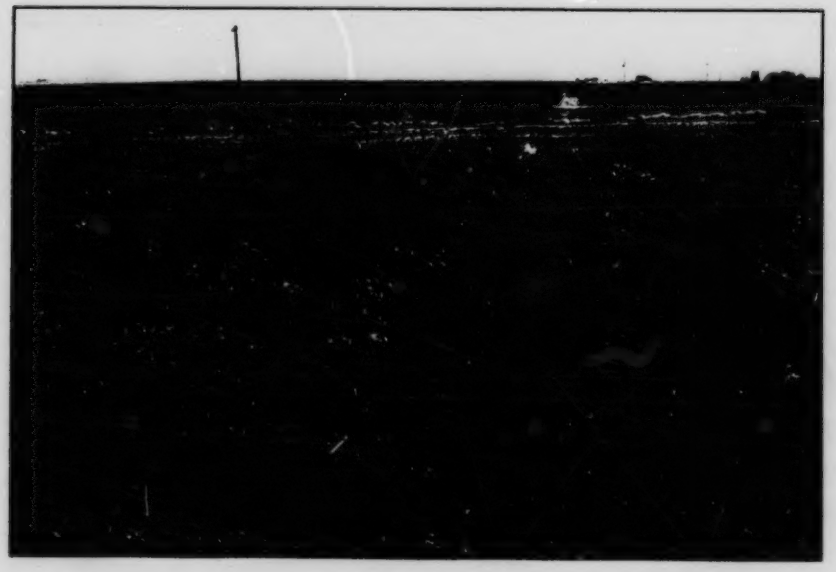


Figure 2.1 Medicinal plot provided by Dr. Peter Sexton and Rhonda Bafus (Technician) of the Central Oregon Agricultural Research Centre, Oregon State University.

3.0 Results

3.1 General Information

3.1.1 Soil Tests

Before the Saskatchewan locations were planted, soil samples were taken and analyzed by Enviro-Test Agricultural Services to determine the N, P, K and S status. Soil analysis indicated that Saskatoon has a clay soil with a pH of 8.2, and was marginal in both NO_3-N and P. Both nutrient levels were increased to 80lbs/ac. La Ronge soil analysis indicated that the soil is clay with a pH of 6.8 and was marginal in both NO_3-N and P. Both nutrients were increased to 80lbs/ac. The Outlook location is loam soil with a pH of 8.0. The NO_3-N and P brought up to 80lbs/ac.

Soil samples were also collected at the Madras, OR site. Samples were collected at 1' and 2' depths. Phosphorous had 8.1ppm and 8.2 ppm for 1' and 2' sampling depths. Potassium was at 423 and 241 ppm, NO_3-N at 11 lbs./ac and 8lbs/ac and sulfur at 6.1 and 5.4 ppm. All nutrients were brought up to the recommended 80lbs/ac of NO_3-N and P.

Table 3.1 Comparison of nutrient availability between all locations for 1999 & 2000.

Year	Site	Available N	Available P	Available K	Available S	Fertilizer Added
1999	*West Lafayette, IN, USA*	unknown	unknown	unknown	unknown	unknown
	Madras, OR, USA	58lbs/ac	95lbs/ac	1640lbs/ac	8lbs/ac	2900g of NH4NO3 and 9375g gypsum
	Davis, CA, USA	unknown	unknown	unknown	unknown	unknown
	Saskatoon, SK, Canada	60lbs/ac	134lbs/ac	1978lbs/a	32lbs/ac	59lbs/ac 34-0-0
	La Ronge, SK Canada	57.8lbs/ac	64.6lbs/ac	1091lbs/ac	61.2lbs.ac	61.6lbs/ac 12-51-0 43lbs/ac 34-0-0
	Outlook, SK, Canada	36lbs/ac	66lbs/ac	610lbs/ac	120lbs/ac	300lbs/ac 5-25-30
2000	*Madras, OR, USA*	24.1lbs/ac	8.2ppm	8ppm	5.4ppm	unknown
	Davis, CA, USA	unknown	unknown	unknown	unknown	unknown
	Saskatoon, SK, Canada	54lbs/ac	140lbs/ac	1432lbs/a	32lbs/ac	62lbs/ac 34-0-0
	La Ronge, SK Canada	62.2lbs/ac	65lbs/ac	1100/ac	58lbs/ac	58lbs/ac 12-51-0 40lbs/ac 34-0-0
	Outlook, SK, Canada	unknown	unknown	unknown	unknown	unknown

3.1.2 Weather Data

1999

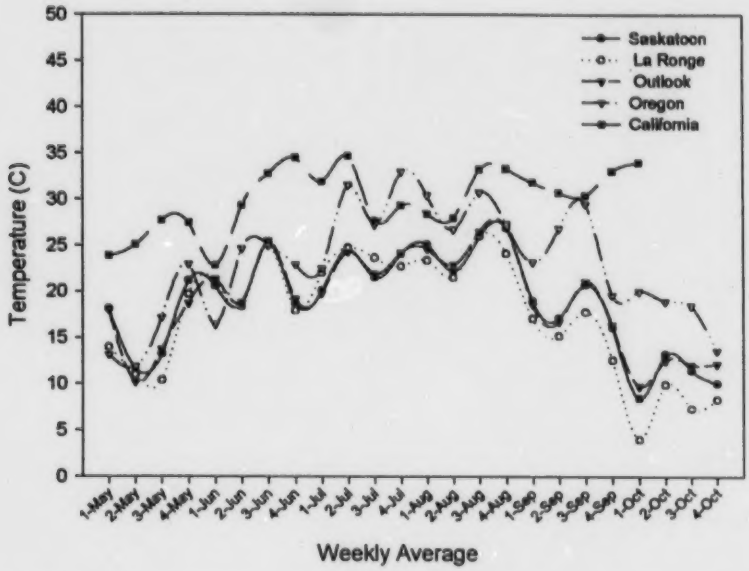


Fig. 3.2 Weekly average maximum temperatures (°C) for Saskatoon, La Ronge, Outlook, Davis, California and Oregon, 1999.

Maximum & Minimum Temperature (℃) and Precipitation (mm) Data

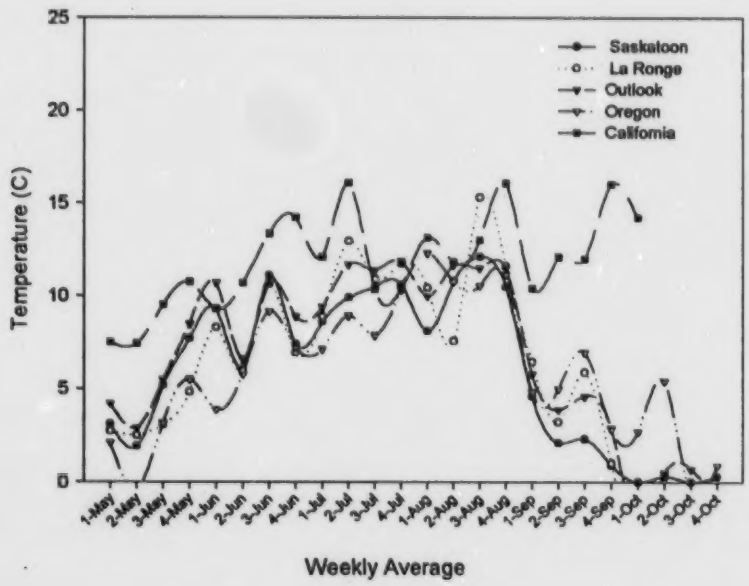


Fig. 3.3 Weekly average minimum temperatures (°C) for Saskatoon, La Ronge, Outlook, Davis California and Oregon 1999.

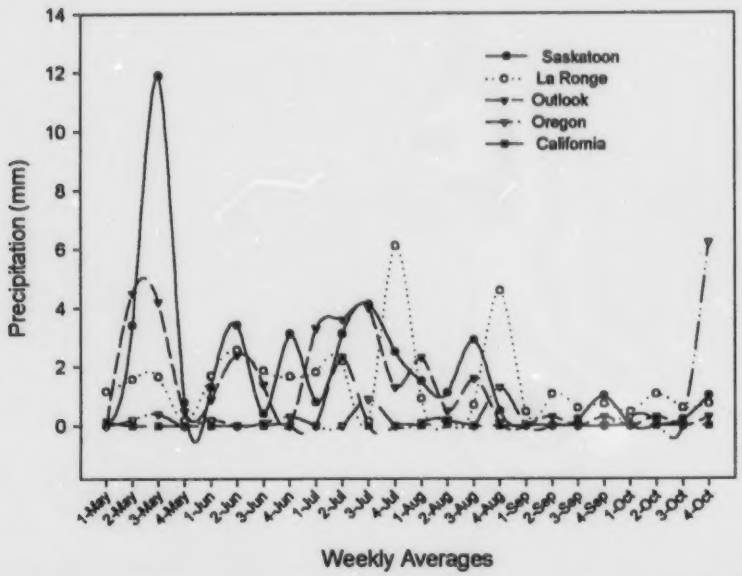


Fig. 3.4 Weekly average precipitation (mm) for Saskatoon, La Ronge, Outlook, Davis California and Oregon, 1999.

2000

Maximum & Minimum Temperatures (°C) and Precipitation (mm) Data

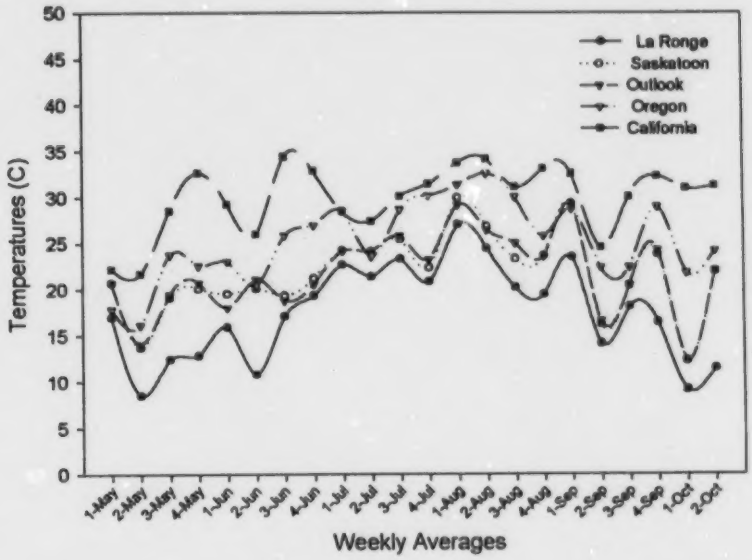


Fig. 3.5 Weekly average maximum temperatures (°C) from May 1st to October 1st 2000.

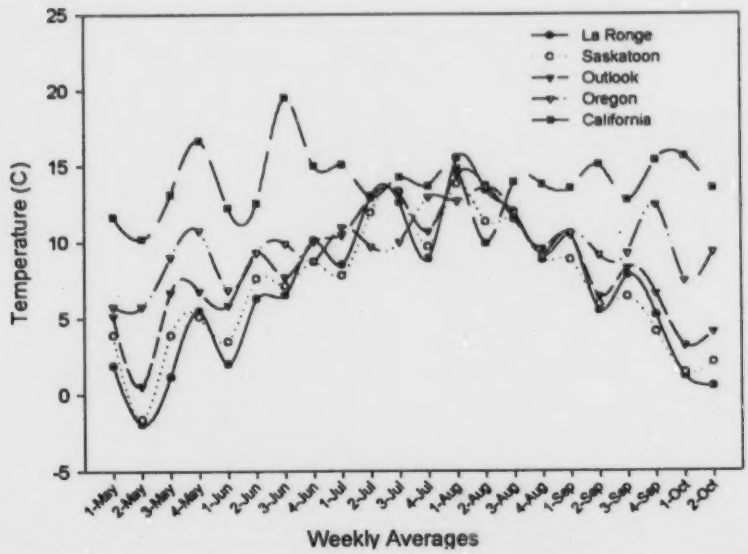


Fig. 3.6 Weekly average of minimum temperatures (°C) from May 1st to October 1st 2000.

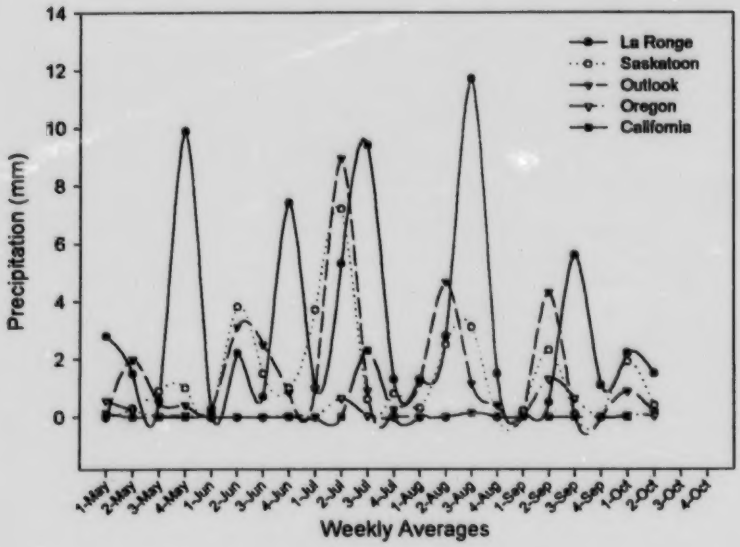


Fig. 3.7 Weekly average precipitation (mm) from May 1st to October 1st 2000.

3.2 *Echinacea angustifolia* (Narrow-leaved Purple Cone-flower)

3.2.1 Improved Germination Methods

An improved germination procedure was developed which increased germination from 26% to 76% (Table 3.2). Echinacea seeds were first treated with a 10% bleach solution and then placed in moist vermiculite in double plastic bags and placed in 4°C-lighted cooler on the 4th of March for stratification. On the 1st of April, stratification was complete and 1/3 of the seeds were planted into seeding trays filled with Sunshine Mix #5 and placed into a 25°C greenhouse for germination. As seeds germinated, they were removed and placed into trays for further propagation. Seedlings were fertilized weekly with five hundred (500mg/L) solution of 20:20:20 (N:P:K).

Table 3.2 Echinacea Germination Experiment

Control	13/50 seeds germinated	26% (no chilling, no bleach)
Bleach	38/50 seeds germinated	76% (4 week chilling + bleach)
No Bleach + Ethephon	36/100 seeds germinated	36% (2 week chilling)
Bleach + Ethephon	23/50 seeds germinated	46% (4week total chilling)

3.2.2 Stand Establishment

Good stand establishment of transplants between 76-97% was reported for most regions except California where crops failed to establish 2 years in a row (1999 and 2000) (Table 3.4). In 1999, Aster yellows affected most Echinacea crops over all locations (Figs. 3.8, 3.9) and in spring 2000, plants were re-established. Second year yields on a limited number of plants were only available for Saskatoon and Outlook sites (Table 3.9).

3.2.3 Harvest Dates

Date to first flowering was uniform across Saskatchewan locations but flowering was delayed in the Oregon crop (Table 3.3). All Saskatchewan crops were harvested in mid October while the Oregon crop was harvested 3-4 weeks later in both years.

Table 3.3 *Echinacea angustifolia* transplant date, date of first flowering and date of harvest for 1999 and 2000.

Year	Site	Transplant Date	Date of First Flowering	Date of Harvest
1999	*West Lafayette, IN, USA*	No data	No data	No data
	Madras, OR, USA	May 18th	October 15th	November 15th
	Davis, CA, USA	Crop Failure	Crop Failure	Crop Failure
	Saskatoon, SK, Canada	May 31st	August 16th	October 20th
	La Ronge, SK, Canada	June 1st	August 17th	October 15th
	Outlook, SK, Canada	June 3rd	August 19th	October, 18th
2000	*Madras, OR, USA*	May 25th	No data	November 3rd
	Davis, CA, USA	Crop Failure	Crop Failure	Crop Failure
	Saskatoon, SK, Canada	June 5th	No data	October 19th
	Outlook, SK, Canada	June 7th	No data	October 20th
	La Ronge, SK, Canada	June 6th	No data	October 23rd

Table 3.4 Percent stand establishment for *Echinacea angustifolia*, 1999 & 2000.

Year	Site	Percentage of Stand Establishment
1999	*West Lafayette, IN, USA*	unknown
	Madras, OR, USA	95.00%
	Davis, CA, USA	Crop Failure
	Saskatoon, SK, Canada	91.26%
	La Ronge, SK, Canada	76.25%
	Outlook, SK, Canada	97.50%
2000	*Madras, OR, USA*	Data not collected
	Davis, CA, USA	
	Saskatoon, SK, Canada	
	Outlook, SK, Canada	
	La Ronge, SK, Canada	

Figure 3.8 Comparison of healthy Echinacea (left) to Echinacea infected with *Aster yellows* (right) from Outlook, SK 1999.

Figure 3.9 *Echinacea angustifolia* flowers which are healthy (left) and *Aster yellows* infected (center and right) in Saskatoon, SK research plots, 1999.

3.2.4 Yield and Future Prospect

Location was a significant yield factor in 1999 when Saskatoon and Outlook sites had 500% greater fresh weight root yield compared to Oregon and La Ronge (Table 3.5). Location was also significant for *Aster Yellows*-infected plants (Table 3.6), however, the disease itself did not reduce root fresh weight below yields from healthy plants. This is an important aspect for growers who might have otherwise plowed up their crops. The year 2000 was not a good growing year for any of the locations and by August, plants were still stunted in growth (Figs. 3.10, 3.11, and 3.12). Nevertheless, significant differences were detected between locations (Table 3.7) with the lowest yield reported at the La Ronge site and the highest yields at the Saskatoon, Outlook and Oregon locations. Saskatoon and Oregon fresh weight root yields were 250% greater than La Ronge. The year 2000 was generally a poor growing season in La Ronge with unseasonably low maximum and minimum temperatures (Figs. 3.5, 3.6). When both years were combined, only the site x year interactions were significant (Tables 3.8). Over all locations, Saskatoon had the most consistently high yields between years. Second year growth was

greater in the Outlook location but variation was high and means were not significantly different from the Saskatoon site (Table 3.9).

Table 3.5 Average yield (g) of healthy first season *Echinacea angustifolia* roots, 1999.

Site Location	Mean Fresh Weight (g) Per Plant	Mean Dry Weight (g) Per Plant
Madras, OR, USA	10.74	‡
Saskatoon, SK, Canada	38.07	12.34
La Ronge, SK, Canada	16.35	4.57
Outlook, SK, Canada	41.93	16.44
Statistical Significance		
Site	**	**
LSD	16.83	7.72

† ** denotes $p \leq 0.01$, ns = not significant
‡ Improper drying for comparison

Table 3.6 Mean dry weight (g) comparison of year old *Echinacea angustifolia* roots infected with *Aster Yellows*, 1999.

Condition	Site Location	Mean Dry Weight (g) Per Plant
Healthy	*Saskatoon, SK, Canada*	12.34
	La Ronge, SK, Canada	4.57
	Outlook, SK, Canada	16.44
Aster yellows	*Saskatoon, SK, Canada*	18.55
	La Ronge, SK, Canada	2.35
	Outlook, SK, Canada	20.55
Statistical Significance		
Site		*
Condition		ns
Site*Condition		ns
LSD		
Site		3.46

† * denotes $p \leq 0.05$; ns = not significant

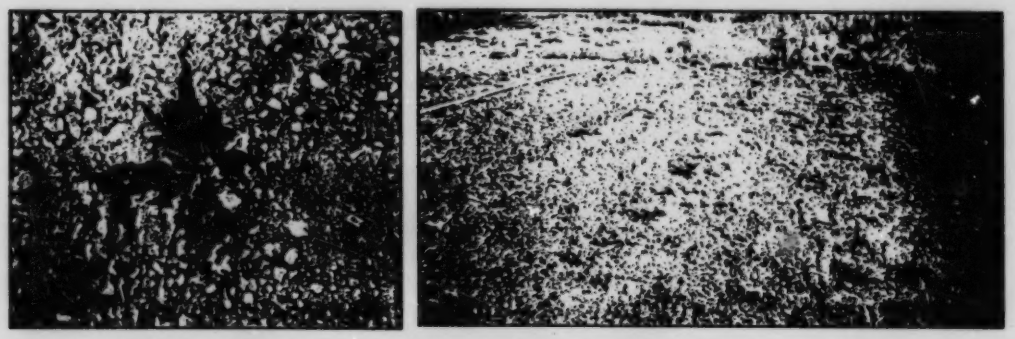


Figure 3.10 La Ronge, SK. Echinacea plot in mid August, 2000.

Figure 3.11 Echinacea plot in Outlook, SK mid August, 2000.

Figure 3.12 Echinacea plant in the Saskatoon, SK research plots on August 3rd, 2000.

Table 3.7 Comparison of healthy *Echinacea angustifolia* mean dry weight (g) per plant for one year old roots in 2000.

Site	Mean Dry Weight (g) Per Plant
Saskatoon, SK, Canada	9.65
La Ronge, SK, Canada	3.45
Outlook, SK, Canada	7.15
Madras, OR, USA	9.03
Statistical Significance	
Location	*
LSD	3.94

† * denotes significance at $p<0.05$

Combined 1999-2000

Table 3.8 Comparison of healthy *Echinacea angustifolia* average per plant fresh weights (g) over two testing periods (1999 & 2000) of one year old roots.

Year	Site Location	Mean Fresh Weight (g) Per Plant	Mean Dry Weight (g) Per Plant
1999	*Saskatoon, SK, Canada*	38.07	12.33
	La Ronge, SK, Canada	13.35	4.57
	Outlook, SK, Canada	41.9	16.44
	Madras, OR, USA	10.74	‡
2000	*Saskatoon, SK, Canada*	28.13	9.65
	La Ronge, SK, Canada	11.28	3.45
	Outlook, SK, Canada	19.83	7.15
	Madras, OR, USA	30.86	‡
Statistical Significance			
Site		ns	ns
Year		ns	ns
Site*Year		**	*
LSD			
Site*Year		16.99	3.61

† ** denotes $p\leq0.01$; ns = not significant
‡ Material not dried properly for comparison

Table 3.9 Comparison of average healthy *Echinacea angustifolia* dry weights (g) per plant for 2001 on two-year-old plant material.

Site	Mean Dry Weight (g) Per Plant
Saskatoon, SK, Canada	29.41
Outlook, SK, Canada	41.08
Statistical Significance	
Location	ns

† ns = not significant

3.3 *Valeriana officinalis* (Valerian)

3.3.1 Germination

Valerian has germination inhibitors in the seed coat and therefore, it is important that seeds are not left soaking in seed coat leachate. Water should be changed on a daily basis. Germination percentages were provided in the initial one year final report.

3.3.2 Transplant and Harvest

Davis, California reported the longest season with transplants on June 1 and harvest on Dec. 23rd in 1999 (Table 3.10). In 2000, both Oregon and California sites had over-wintered plants. In Saskatchewan, all harvests occurred between the 2nd or 3rd week of October.

3.10 Valeriana officinalis (Valerian) transplant and harvest dates for all test sites in 1999 and 2000.

Year	Site	Transplant Date	Date of Harvest
1999	*West Lafayette, IN, USA*	No Data	No Data
	Madras, OR, USA	May 18th	November 15th
	Davis, CA, USA	June 1st	December 23rd
	Saskatoon, SK, Canada	May 31st	October 20th
	Outlook, SK, Canada	June 3rd	October 18th
	La Ronge, SK, Canada	June 1st	October 15th
2000	*Madras, OR, USA*	Second year planting	November 3rd
	Davis, CA, USA	Second year planting	November 29th
	Saskatoon, SK, Canada	June 5th	October 19th
	Outlook, SK, Canada	June 7th	October 20th
	La Ronge, SK, Canada	June 6th	October 23rd

3.3.3 Yield and Future Prospect

In 1999, there was a significant effect of disease level (Figs. 3.13, 3.14, 3.15, 3.16) on root yield. Advanced stages were 9 times lower in root yield compared to early stage (stage 3, slight leaf discolouration) over all Saskatchewan sites (Table 3.14). Even low incident of disease significantly reduced root fresh weight in La Ronge compared to Outlook and Saskatoon sites (Table 3.12). There was a significant site x condition interaction and the Saskatoon location at the same disease stage still had greater dry weight compared to the La Ronge and Outlook locations (Tables 3.12, 3.13). In 2000, there was no significant difference in the average dry weights of valerian root yield in healthy plants between locations (Table 3.15). However, there was again a significant site x condition interaction and the Saskatoon location at the same disease stage still had greater dry weight compared to the La Ronge and Outlook locations (Table 3.16). For all sites, the advanced stage had significantly lower mean dry weight compared to less developed stages (Table 3.17). Until Aster yellows can be managed, this disease poses a significant constraint to valerian crop expansion in this province.

Fig 3.13 Healthy valerian plant in Outlook, SK plot 1999.

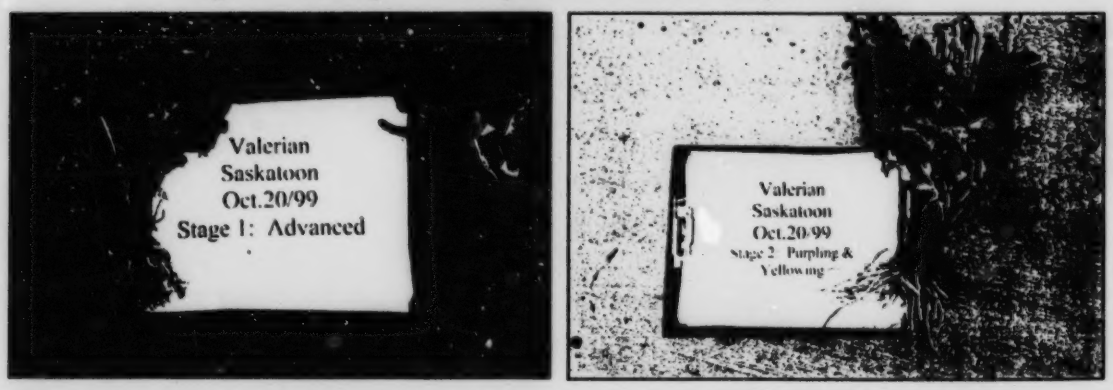


Fig. 3.14 Stage one of valerian infected with aster yellows (left). Stage two infection of valerian (purpling & yellowing of leaves (right) 1999,

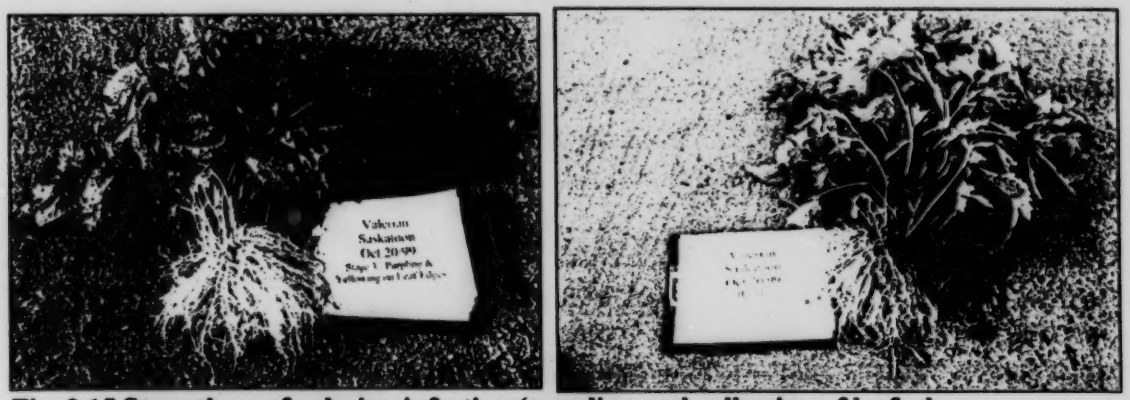


Fig. 3.15 Stage three of valerian infection (purpling and yellowing of leaf edges – slight discoloration) – left. A healthy valerian plant –right.

Table 3.11 Comparison of healthy valerian fresh weight (g) per plant root samples between all growing locations in 1999.

Site	Healthy Valerian Root Mean Fresh Weight
West Lafayette, IN, USA	‡
Madras, OR, USA	353.5
Davis, CA, USA	841.1
Saskatoon, SK, Canada	611.2
La Ronge, SK, Canada	783.9
Outlook, SK, Canada	370.7
Statistical Significance	
Site	*
LSD	263.00

† *denotes $p \leq 0.05$
1 Means are based on four replicates
‡ No plant material received.

Table 3.12 Site and disease stage interaction between the Saskatchewan locations in 1999.

Site	Fresh Weight (g) Per Plant		
Disease Stage	***Stage1 (Advanced)***	***Stage 2 (Moderate Damage with Yellowing & Purpling of Leaves)***	***Stage 3 (Early Stage of Infection with Slight Leaf Discoloration)***
Saskatoon, SK, Canada	27.87	149.63	436.28
La Ronge, SK, Canada	53.71	99.40	145.78
Outlook, SK, Canada	24.73	194.78	373.75
Statistical Significance			
Site* Disease Stage		*	
LSD			
Site*Disease Stage		98.69	

† *denotes $p \leq 0.05$

Table 3.13 Site interaction between the Saskatchewan locations for infected plant material, 1999.

Site	Fresh Weight (g) Per Plant
Saskatoon, SK, Canada	204.59
La Ronge, SK, Canada	99.63
Outlook, SK, Canada	197.75
Statistical Significance	
Site	*
LSD	
Site	56.98

† *denotes $p \leq 0.05$

Table 3.14 Comparison between the various stages of infection

Level of Disease Development	Fresh Weight (g) Per Plant
Stage 1 (Advanced)	35.43
Stage 2 (Moderate Damage of Yellowing & Purpling of the Leaves)	147.93
Stage 3 (Early Stage of Infection with Slight Leaf Discoloration)	318.60
Statistical Significance	
Disease Development	*
LSD	
Disease Development	56.98

† *denotes $p \leq 0.05$

In Saskatoon, the plots were kept to study the effect of *Aster yellows* on the second year of an infected crop. Initial plant growth indicated little presence of the disease until warmer weather in May. Visible signs of Aster yellows (yellowing of tissue, deformed leaves and plant die back) occurred in the latter part of May. Due to the severity of the infection, the plant material was dug up and destroyed before the new crop was planted to prevent further infection in other crops. No data was therefore collected on the second year plot.

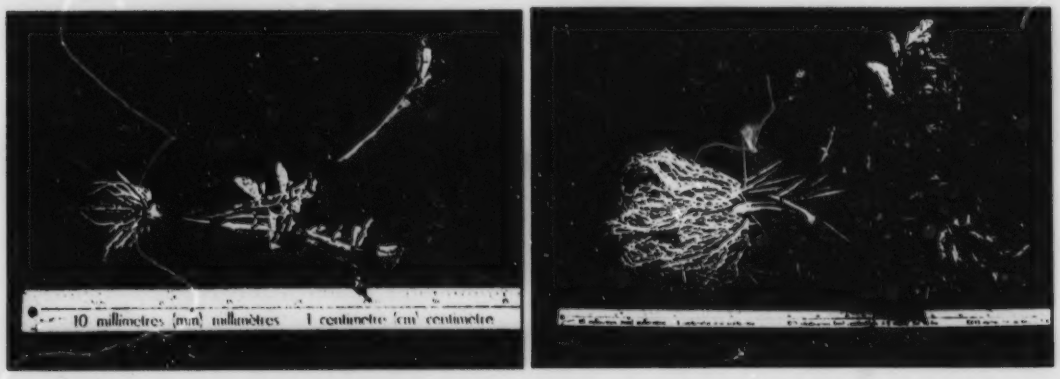


Fig. 3.16 One year old Valerian in Outlook infected with Aster yellows at the stage 1 (left) and stage 3 (right) in October 2000.

Table 3.15 Comparison of one year old, healthy valerian dry weight (g) per plant root samples at all growing locations in 2000.

Site	Mean Dry Weight (g) Per Plant
Saskatoon, SK, Canada	175.0
La Ronge, SK, Canada	210.0
Outlook, SK, Canada	225.0
Davis, CA, USA	196.9
Statistica. Significance	
Location	ns
† ns = not significant	

Table 3.16 Site interaction between the Saskatchewan locations for infected plant material, 2000.

Site	Mean Dry Weight (g) Per Plant		
Level of Disease	***Stage1 (Advanced)***	***Stage 2 (Moderate Damage with Yellowing & Purpling of Leaves)***	***Stage 3 (Early Stage of Infection with Slight Leaf Discoloration)***
Saskatoon, SK, Canada	61.5	158.7	213.7
La Ronge, SK, Canada	4.8	27.3	51.0
Outlook, SK, Canada	21.0	101.2	172.5
Statistical Significance			
Site*Level of Disease		*	
LSD		36.97	
† * denotes p≤0.05			

Table 3.17Comparison between the various stages of infection

Disease Level	Mean Dry Weight (g) Per Plant
Stage 1 (Advanced)	175.0
Stage 2 (Moderate Damage of Yellowing & Purpling of the Leaves)	210.0
Stage 3 (Early Stage of Infection with Slight Leaf Discoloration)	225.0
Statistical Significance	
Disease	*
LSD	21.39
† * denotes p≤0.05	

3.4 Arctium lappa (Burdock)

3.4.1 Germination

The burdock was not germinated within the greenhouse. Instead, seed was direct seeded due to information of 100% germination. Burdock was planted in Saskatoon on the 31st of May. La Ronge and Outlook plots were planted on the 2nd and 3rd of June, respectively. Seeds were placed at a 60cm (24") inter-row spacing with a 1.2m between row spacing. A 5% germination rate occurred; therefore all six plots discontinued the burdock portion of the project for the 1999-year. The Oregon, California and Indiana locations also experienced similar germination problems by direct seeding. Burdock transplants were used in 2000. However, volunteer burdock plants were also observed in the spring and fall seeding should be considered in future work.

3.4.2 Harvest Dates

Most locations planted and harvested burdock within one week of each other early June transplants and third week in October harvest (Table 3.18). All burdock at all sites were hand dug with the whole root system extracted. Oregon had the longest growing season transplanting May 25 and harvesting November 3

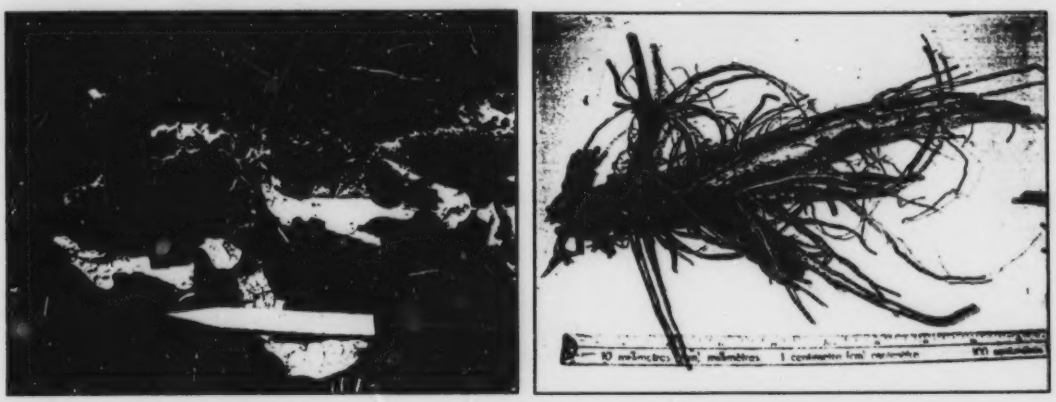


Fig. 3.17 *Arctium lappa* in the field at Saskatoon on August 3rd (left) and harvested roots from Outlook on October 20th (right).

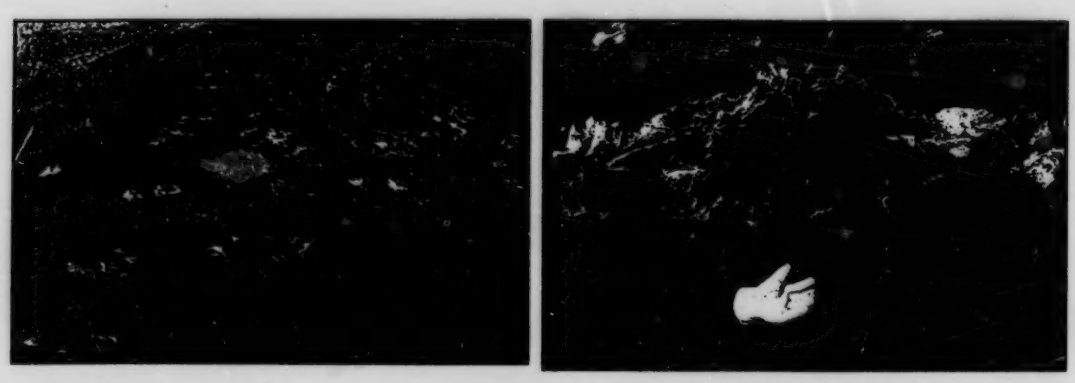


Fig. 3.18 Burdock plot (left) and plant (right) at the research plots in Madras, Oregon USA 2000.

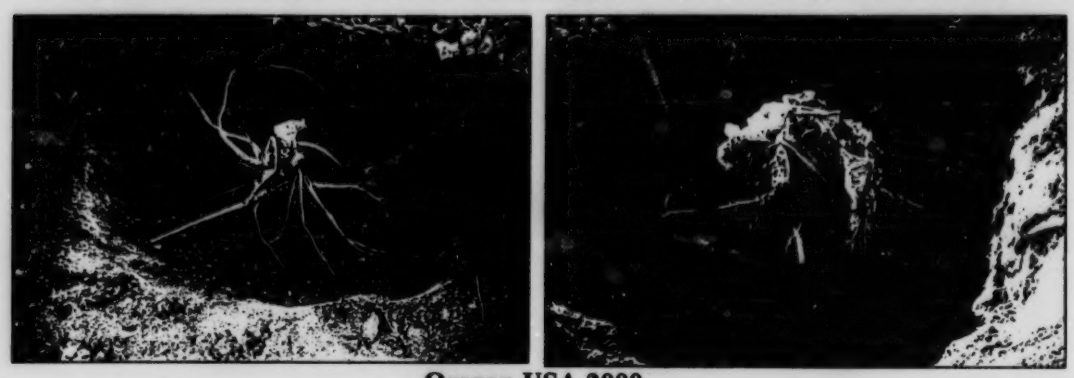


Fig. 3.19 Rooting system of a single burdock plant in Saskatoon, SK, on October 2nd 2000.

3.4.3 Yield and Future Prospect

In spite of the long growing season and the large plants (Fig. 3.18), Oregon had the lowest per plant dry weight (Table 3.19). There was no significant difference in dry weight yield between Saskatchewan and Davis, California sites. Constraints to future crop expansion include development of mechanical harvesting equipment to harvest the roots in that roots are found about 3 feet below ground (Fig. 3.19).

Table 3.18 Transplanting and harvesting dates of *Arctium lappa* at all experimental sites in 2000.

Site	Transplant Date	Date of Harvest
Madras, OR, USA	May 25th	November 3rd
Davis, CA, USA	June 2nd	October 23rd
Saskatoon, SK, Canada	June 5th	October 19th
Outlook, SK, Canada	June 7th	October 20th
La Ronge, SK, Canada	June 6th	October 24th

Table 3.19 Comparison of *Arctium lappa* average per plant dry weights (kg) between locations in 2000.

Site	Mean Dry Weight (kg) Per Plant
La Ronge, SK, Canada	0.15
Davis, CA, USA	0.22
Outlook, SK, Canada	0.20
Saskatoon, SK, Canada	0.15
Madras, OR, USA	0.04
Statistical Significance	
Location	**
LSD	0.10

† ** denotes $p \leq 0.01$

3.5 *Nepeta cataria* (Catnip)

Fig. 3.20 Catnip crop, Saskatoon location just prior to harvest in 1999.

3.5.1 Seed Germination

In Davis, CA, it was reported that the best method to germinate catnip was to spread it directly into the trays containing a potting mixture and then lightly spread a layer of soil over it. Germination of the seeds occurred within one week.

3.5.2 Stand Establishment

Most locations reported 100% establishment in both years after transplanting (Table 3.21). However, in the second year of the study difficulties with catnip germination were reported in Davis, CA and only 30% of the seed germinated. On the 27th of June, 2000 difficulties were still reported. Three plantings had been initiated but the seedlings would germinate and then remain in their cotyledon stage for 4 weeks. The transplants were moved outside to see if growth could be increased but due to high temperatures, many of the new seedlings were lost.

Saskatoon location: Seedlings turned entirely purple once planted, however, after a week in the field, the plants greened up and grew vigorously. Soil moisture levels were monitored and watering occurred as necessary. Later in the summer a few of the catnip plants in Saskatoon and La Ronge began to again turn purple. In this instance, it was not because of a fertility problem as the colour change was concentrated on the lower stems close to the main stalk. Dr. Jill Thomson, Plant Pathologist for the Department of Plant Sciences analyzed the plant material. On initial inspection and plating of the tissue, it was determined that *Rhizoctonia* was a possibility, however further testing would be necessary for an accurate determination. Those plants with disease symptoms were removed from the plot.

3.5.3 Harvest Dates

Once >80% of the floral spikes were in bloom, harvesting was performed. Harvest dates were 3-4 weeks earlier in Oregon in both years even though planting dates were within one week apart between all sites (Table 3.20). The Oregon location had consistently higher maximum temperatures during early, mid and late growing season (Figs. 3.2, 3.5) compared to Saskatchewan sites which likely advanced growth in this crop. Flowering date was earliest in Oregon and latest in La Ronge. La Ronge also had the latest harvest (August 18-31). Harvesting occurred on August 23rd in Saskatoon and Outlook. While Davis, California had the highest overall temperatures, members of the mint family normally grow best under cooler temperatures.

Catnip was over-wintered in Madras, OR in 1999/2000. On the 28th of April 2000, the plants were approximately 20-30" tall and vigorous. By the 9th of May, the catnip plants were 24-36" tall. In spite of the advance in development in the overwintered Oregon crop, harvest occurred on the 31st of July, four days after the 1999 harvest.

3.5.4 Yield and Future Prospect

Year x Location were significant (Table 3.24). In 1999, Saskatoon and Outlook had significantly higher fresh (Table 3.22) and dry weights than all other U.S. and La Ronge locations. However, Oregon and Saskatoon expressed the greatest dry weight yield in 2000 (Table 3.23). The Saskatoon location had the most consistently higher

yield over all locations between both years with 48% to 300% greater dry weight of Saskatoon-grown plants over Davis, California and all other Saskatchewan sites and therefore is worthy of further investigation.

Table 3.20 *Nepeta cataria* (Catnip) transplant, first flowering and date of harvest for catnip grown at various Canadian and USA sites in 1999 and 2000.

Year	Site	Transplant Date	Date of First Flowering	Date of Harvest
1999	*West Lafayette, IN, USA*	May 26th	July 15th	July 22nd
	Madras, OR, USA	May 24th	June 30th	July 27th
	Davis, CA, USA	June, 6th	July 17th	August 18th
	Saskatoon, SK, Canada	May 31st	July 1st	August 25th
	La Ronge, SK, Canada	June 1st	July 1st	August 18th
	Outlook, SK, Canada	June 3rd	July 14th	August, 20th
2000	*Madras, OR, USA*	Second year stand	Unknown	July 31st
	Davis, CA, USA	June 2nd	Unknown	August 23rd
	Saskatoon, SK, Canada	June 5th	July 12th	August 23rd
	Outlook, SK, Canada	June 7th	July 3rd	August 23rd
	La Ronge, SK, Canada	June 6th	July 24th	August 31st

Table 3.21 Percent stand establishment for *Nepeta cataria*, 1999 & 2000.

Year	Site	Percent Stand Establishment
1999	*West Lafayette, IN, USA*	unknown
	Madras, OR, USA	100%
	Davis, CA, USA	100%
	Saskatoon, SK, Canada	100%
	La Ronge, SK, Canada	100%
	Outlook, SK, Canada	100%
2000	*Madras, OR, USA*	Second year stand
	Davis, CA, USA	100%
	Saskatoon, SK, Canada	100%
	La Ronge, SK, Canada	100%
	Outlook, SK, Canada	100%

Table 3.22 Comparison of catnip average yields (kg) on a per plant basis in 1999.

Site Location	Fresh Weight Mean (kg) Per Plant	Dry Weight Mean (kg) Per Plant
West Lafayette, IN, USA	0.88	‡
Madras, OR, USA	0.72	‡
Davis, CA, USA	1.33	0.25
Saskatoon, SK, Canada	1.51	0.37
La Ronge, SK, Canada	0.91	0.26
Outlook, SK, Canada	1.59	0.31
Statistical Significance		
Site	**	**
LSD	0.47	0.09

† ** denotes $p \leq 0.01$

‡ Weights improperly dried and unable to include.

Fig. 3.21 Flower stalks of catnip in the Saskatoon research plots on August 12th, 1999.

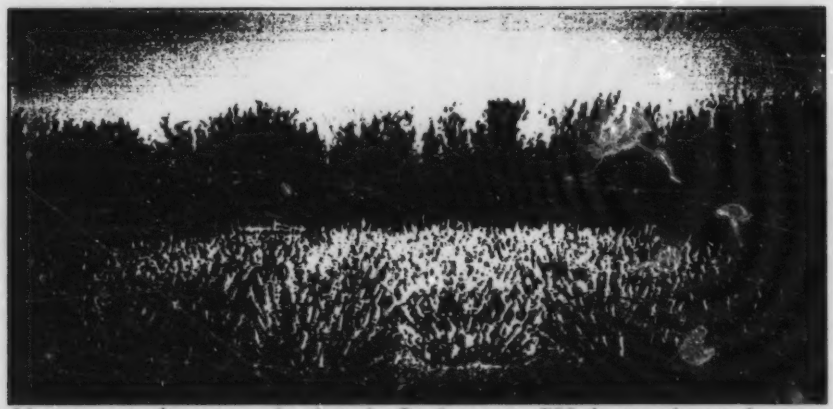


Fig. 3.22 *Nepeta cataria* research plots in Saskatoon, SK. just prior to harvesting in the fall of 1999.

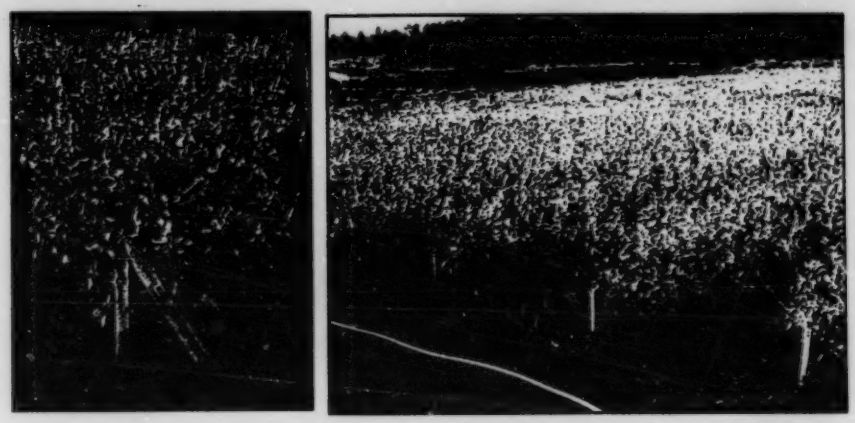


Fig. 3.23 *Nepeta cataria* research plots at Saskatoon on August 3rd, 2000.

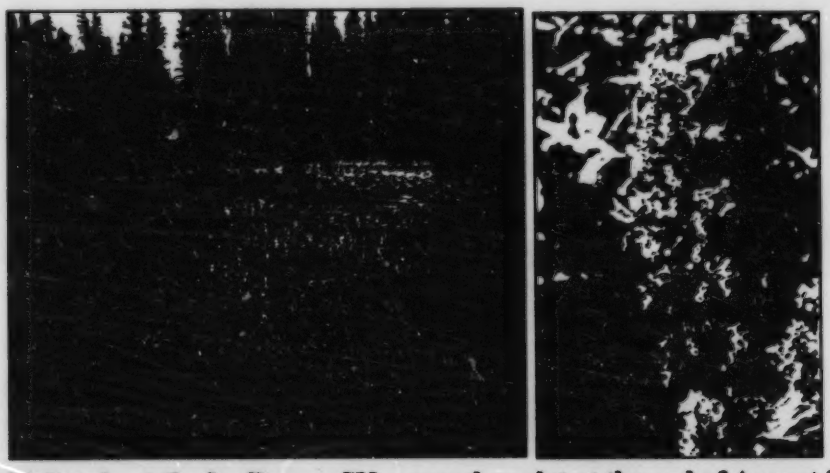


Fig. 3.24 Catnip at the La Ronge, SK researches plot at the end of August 2000.

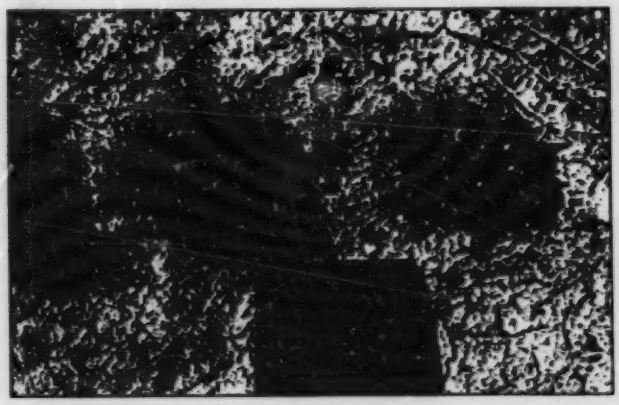


Fig. 3.25 Over-wintered *Nepeta cataria* at Madras, Oregon 2000.

Table 3.23 Comparison of *Nepeta cataria* average dry weight (kg) on a per plant basis in 2000.

Site	Mean Dry Weight (kg) Per Plant
+*Madras, OR, USA*	0.47
Saskatoon, SK, Canada	0.44
La Ronge, SK, Canada	0.18
Outlook, SK, Canada	0.17
Davis, CA, USA	0.14
Statistical Significance	
Location	*
LSD	0.22

†* denotes significance at $p<0.05$.
+ plant material two years old.

Combined Analysis

Table 3.24Comparison of *Nepeta cataria* average yields (kg) per plant over two years (1999 & 2000).

Year	Site	Mean Fresh Weight (kg) Per Plant	Mean Dry Weight (kg) Per Plant
1999	*Davis, CA USA*	1.33	0.25
	Saskatoon, SK, Canada	1.51	0.37
	La Ronge, SK, Canada	0.91	0.26
	Outlook, SK, Canada	1.59	0.31
	Madras, OR USA	0.72	‡
2000	*Davis, CA USA*	0.61	0.14
	Saskatoon, SK, Canada	1.17	0.44
	La Ronge, SK, Canada	0.63	0.18
	Outlook, SK, Canada	0.59	0.17
	Madras, OR USA	0.65	0.47
Statistical Significance			
Year		ns	ns
Location		ns	ns
Year*Location		**	**
LSD			
Year*Location		0.36	0.07

† ** denotes $p\leq0.01$, ns = not significant
‡ Weights improperly dried and unable to include.

3.6 *Calendula officinalis* (Calendula)

3.6.1 Germination

High germination percentage and rates generally characterize this crop. Most crops germinated within 7 days.

3.6.2 Stand Establishment

Calendula expressed 100% stand establishment after transplanting in both years and over all sites reporting data (Table 3.26).

3.6.3 Harvest Dates

Planting dates were similar, however, date of first harvest ranged between years and sites except for Davis, CA where first harvest consistently occurred on July 17 (Table 3.25).

Saskatchewan sites in 1999 began harvest in late June. In 2000, harvest did not commence until late July/early August. Unseasonable cool maximum and minimum temperatures in 2000 (Figs. 3.5, 3.6) compared to 1999 (Figs. 3.2, 3.3) likely caused this extreme delay, particularly in La Ronge. End of harvest dates varied widely between years for all locations. For example in 1999, end of harvest in La Ronge occurred after the 4th week of September and in 2000, harvest terminated one month earlier. By contrast, end of harvest in 1999 in Saskatoon occurred after the 4th week of August while in 2000, it occurred one month later (Tables 3.28, 3.29).

Table 3.25 Calendula officinalis (Pot Marigold) transplanting, flowering and harvesting dates for all sites in 1999 and 2000.

Year	Site	Transplant Date	Date of First Flowering	Date of First Harvest
1999	*West Lafayette, IN, USA*	May 26th	July 5th	July 16th
	Madras, OR, USA	May 17th	June 29th	June 29th
	Davis, CA, USA	June 1st	June 10th	July 17th
	Saskatoon, SK, Canada	May 31st	May 31st	June 25th
	La Ronge, SK, Canada	June 1st	June 1st	June 29th
	Outlook, SK, Canada	June 3rd	June 3rd	June 29th
2000	*Madras, OR, USA*	May 9th	Unknown	June 23rd
	Davis, CA, USA	June 2nd	Unknown	July 17th
	Saskatoon, SK, Canada	June 5th	July 11th, 2000	July 25th
	Outlook, SK, Canada	June 7th	July 8th, 2000	July 24th
	La Ronge, SK, Canada	June 6th	July 19th, 2000	August 8th

Table 3.26 Percent stand establishment for *Calendula officinalis*, 1999 & 2000.

Year	Site	Percentage of Stand Establishment
1999	*West Lafayette, IN, USA*	unknown
	Madras, OR, USA	100%
	Davis, CA, USA	100%
	Saskatoon, SK, Canada	100%
	La Ronge, SK, Canada	100%
	Outlook, SK, Canada	100%
2000	*Madras, OR, USA*	100%
	Davis, CA, USA	100%
	Saskatoon, SK, Canada	100%
	La Ronge, SK, Canada	100%
	Outlook, SK, Canada	100%

3.6.4 Yield and Future Prospect

Location, date of harvest and their interactions were all significant in 1999 (Table 3.28). Oregon had the highest mean/week yields followed by Indiana, La Ronge, and Davis CA. Saskatoon and Outlook had the lowest mean fresh weight of calendula flowers of all sites that year (Table 3.27). Aster yellows severely affected the Saskatchewan crops in both years but particularly 1999 (Figs. 3.27, 3.29) and only non-infected flowers were harvested. This had a negative influence on Saskatchewan yields. Until this disease is managed, calendula floral yields will continue to be disadvantaged in this province. In 2000, only the interaction was significant (Table 3.29). That year, Davis California had the highest dry weight yield while La Ronge and Oregon locations had the lowest mean per week yield (Table 3.30). Calendula is adapted to full-sun and high seasonal temperature conditions normally found in Oregon and California (Figs. 3.2, 3.5). However, extreme heat will cause reduced floral size and reduced floral longevity. Evidence of aster yellows in the California and Oregon samples may have also affected

Fig. 3.26 Outlook, SK calendula research plot in 1999.

their yields in various years. Unless the phytochemical components can significantly compensate for Saskatchewan's lower floral biomass yield and until Aster yellows is better controlled, growing calendula in this province may be difficult in terms of competition with U.S. locations.

Fig. 3.27 Calendula flowers, which have Aster yellows (left and centre), compared to a healthy blossom (right).

Table 3.27 Weekly averages of *Calendula officinalis* fresh weights (g) for 1999.

Site	Mean Fresh Weight (g) Harvested Per Week
West Lafayette, IN, USA	273.90 b †
Madras, OR, USA	338.23 a
Davis, CA, USA	172.56 d
Saskatoon, SK, Canada	101.94 e
La Ronge, SK, Canada	230.12 c
Outlook, SK, Canada	129.06 e

† Mean separation for site calculated by the Duncan's Multiple Range Test, 5% level.

Table 3.28 Comparison of the location by date of harvest interaction for *Calendula officinalis* in 1999.

Location	Month	Week of Harvest	Mean Fresh Weight (g)
West Lafayette, IN, USA	July	1	538.80
		2	212.17
		3	No Data
		4	No Data
Madras, OR, USA		1	64.50
		2	393.82
		3	432.00
		4	745.33
Davis, CA, USA		1	71.51
		2	233.25
		3	No Data
		4	No Data
Saskatoon, SK, Canada		1	23.83
		2	30.90
		3	116.33
		4	243.26
La Ronge, SK, Canada		1	11.58
		2	38.58
		3	117.03
		4	367.52
Outlook, SK, Canada		1	33.08
		2	95.01
		3	97.15
		4	293.83
West Lafayette, IN, USA	August	1	71.03
		2	End of Harvest
		3	-
		4	-
Madras, OR, USA		1	728.12
		2	562.13
		3	426.67
		4	283.93
Davis, CA, USA		1	213.50
		2	282.75
		3	433.75
		4	No Data
Saskatoon, SK, Canada		1	151.63
		2	114.78
		3	65.00
		4	47.53

La Ronge, SK, Canada		1	398.68
		2	497.42
		3	502.47
		4	247.55
Outlook, SK, Canada		1	230.18
		2	135.00
		3	301.85
		4	43.75
West Lafayette, IN, USA	September	1	-
		2	-
		3	-
		4	-
Madras, OR, USA		1	243.00
		2	191.13
		3	327.15
		4	298.35
Davis, CA, USA		1	51.85
		2	49.08
		3	44.80
		4	End of Harvest
Saskatoon, SK, Canada		1	End of Harvest
		2	-
		3	-
		4	-
La Ronge, SK, Canada		1	232.8
		2	209.73
		3	130.55
		4	43.28
Outlook, SK, Canada		1	End of Harvest
Statistical Significance			
Location			**
Date of Harvest			**
Location*Date of Harvest			**
LSD			
Location			31.54
Date of Harvest			55.61
Location*Date of Harvest			73.77

† ** denotes $p \leq 0.01$

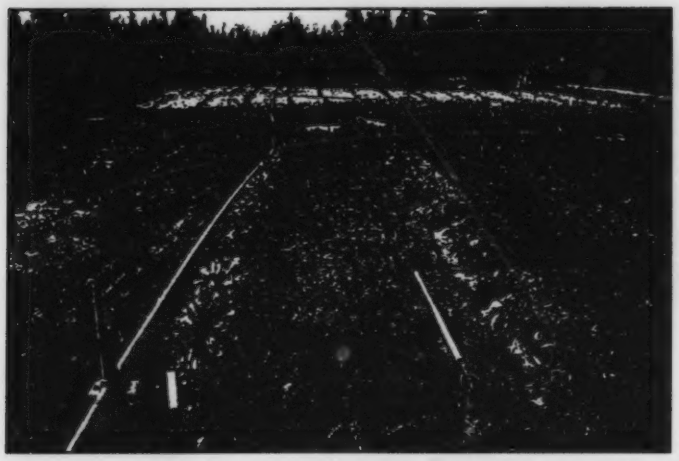


Fig. 3.28 La Ronge, SK calendula research plot June 28th, 2000.

Fig. 3.29 *Aster yellows* shown by the yellowing of the foliage and the apparent venation on the leaves, 2000.

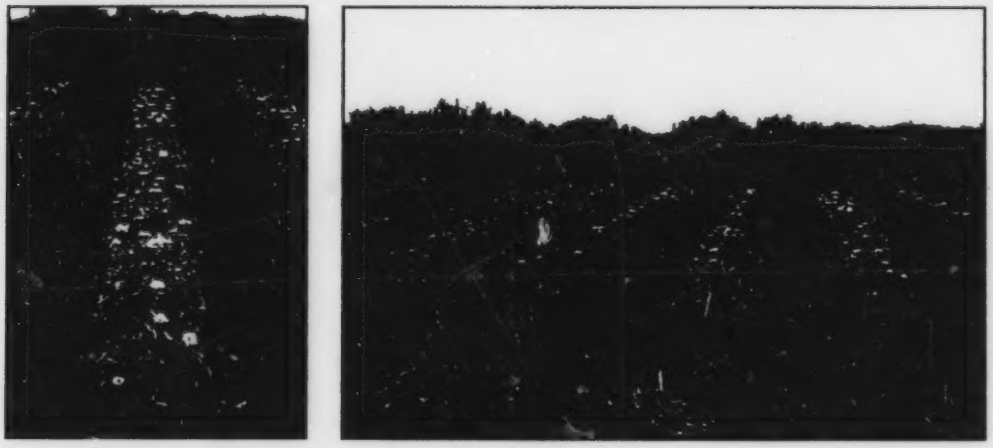


Fig. 3.30 Outlook, SK calendula research plot on July 20th 2000.

Table 3.29 Comparison of the location by date of harvest interaction for *Calendula officinalis* in 2000.

Location	Month	Week of Harvest	Mean Dry Weight (g)
Madras, OR, USA	July	1	18.00
		2	27.95
		3	42.23
		4	17.38
Davis, CA, USA		1	
		2	
		3	Start of Harvest
		4	14.75
Saskatoon, SK, Canada		1	
		2	
		3	
		4	Start of Harvest
La Ronge, SK, Canada		1	Start of Harvest
		2	17.68
		3	21.15
		4	26.33
Outlook, SK, Canada		1	
		2	
		3	Start of Harvest
		4	5.67
Madras, OR, USA	August	1	2.95
		2	7.35
		3	8.35
		4	9.93
Davis, CA, USA		1	39.75
		2	28.25
		3	32.50
		4	42.25
Saskatoon, SK, Canada		1	14.10
		2	25.73
		3	43.13
		4	20.95
La Ronge, SK, Canada		1	7.3
		2	11.6
		3	11.5
		4	16.5
Outlook, SK, Canada		1	14.95
		2	28.23
		3	13.98
		4	37.65
Madras, OR, USA	September	1	18.27

		2	21.78
		3	23.0
		4	18.9
Davis, CA, USA		1	14.00
		2	18.5
		3	14.75
		4	6.5
Saskatoon, SK, Canada		1	12.13
		2	17.35
		3	43.13
		4	5.05
La Ronge, SK, Canada		1	End of Harvest
		2	
		3	
		4	
Outlook, SK, Canada		1	26.25
		2	13.08
		3	12.28
		4	22.85
Statistical Significance			
Location			ns
Date of Harvest			ns
Location*Date of Harvest			**
LSD			
Location			
Date of Harvest			
Location*Date of Harvest			10.36

† ** denotes $p \leq 0.01$

Table 3.30 Weekly averages of *Calendula officinalis* dry weights (g) for 2000.

Site	Mean Fresh Weight (g) Harvested Per Week
Davis, CA, USA	23.47 a†
Saskatoon, SK, Canada	19.78 b
Outlook, SK, Canada	19.44 b
Madras, OR, USA	16.41 c
La Ronge, SK, Canada	15.94 c

† Mean separation for site calculated by the Duncan's Multiple Range Test, 5% level.

3.7 Sily*bum marianum* L. (Milk Thistle)

The objective of this study was to determine if seed source significantly influenced harvestable seed yield or silymarin concentration. Four seed sources were compared: Johnny's Selected Seeds, Maine; Horizon Herbs, Oregon; Richters, Ontario; and a Bulgarian seed source.

3.7.1 Germination

For the purposes of this study, transplants were used, however, fall seeding may have potential since many volunteer seedlings in the spring are normally observed. After transplanting, establishment was generally high with few re-plants required.

3.7.2 Transplant date and flowering

Time to flower was similar between seed sources. Transplants from Richters seeds were delayed but days to first flower were similar to the other seed sources (Table 3.31).

3.7.3 Harvest

All crops were harvested at the same stage of development, at the mature stage (Figs. 3.31 – 3.35). Therefore, seed yield and silymarin content were assessed at the same seed maturity. Visually, little difference was observed in seed morphology between the seed sources (Fig. 3.36).

3.7.4 Yield and Future Prospects

The Bulgarian source produced the largest quantity seed yield with a mean of 28.07 g/plant compared to 11.71 g/plant for Horizon Herbs seed and 12.9 g/ for Johnny's Seed (Table 3.32). At a standard commercial row spacing of 50 cm and 30 cm within row, the yield is equivalent to: 1871.7 kg/ha (Bulgarian source), 782.3 kg/ha (Horizon Herbs), and 860.3 kg/ha (Johnny's Selected Seeds). Yields from more southern regions

are reported to range from 1200 – 1500 kg/ha (*Silybum marianum* info. sheet, Herb Research Program, 1997) and therefore, the Bulgarian source was superior in yield to either the other sourced-seed or yield from southern regions. The Bulgarian seed source produced the earliest yield even though it was planted at the same time as Horizon Herbs and Johnny's Selected Seeds. A recent report (Martin *et al.*, 2000, Proc. 13th Ann. Conf. Agronomy 30:1-5) indicates duration from first emergence to first visible bud was not simply related to thermal time alone but modified by daylength. Duration to first bud required 2000 degree days > 5°C up to 13 hr photoperiod but required fewer degree days as daylength increased. There is likely a cultivar-dependent variation in the requirement of both photo-thermal time to induce flowering. The Bulgarian source appears to be well adapted to Saskatchewan conditions. Other factors such as nitrogen and phosphorus fertility will influence yield (Omer, *et al.*, 1998, Egyptian J. Hort. 25(3):281-293) and may be cultivar-dependent.

Harvest date was also significantly different. Five harvests were conducted weekly between September 5 – October 4 and in all 3 sources, September 26 was the date with the highest seed yield (Table 3.32). Milk thistle is a crop that can withstand several light frosts without visible injury to either the vegetative or reproductive structures. Nevertheless in future, earlier staggered planting dates and fall seeding trials should be examined. With projected global climate change (Table 3.38, *From: Wheaton, E. (2001). Changing Climates: Exploring Possible Future Climates of the Canadian Prairie Provinces. Saskatchewan Research Council, Saskatoon, SK.*), the Saskatchewan season may extend into the fall and collectively renders growing crops such as milk thistle more feasible. Moreover, seeding and harvesting equipment can be easily modified for this crop and mechanical cleaning equipment already exists. If silymarin content does not decrease with frost, milk thistle represents a viable crop for Saskatchewan.

Table 3.31 *Silybum marianum* L. transplanting and flowering dates in Saskatoon, SK. in 2001.

Year	Seed Source	Transplant Date	Date of First Flowering
2001	*Johnny's Selected Seeds, Maine*	June 5th	August 2nd
	Horizon Herbs, Oregon	June 5th	August 2nd
	Richter's, Ontario	June 28th	September 3rd
	Bulgaria	June 5th	July 30th

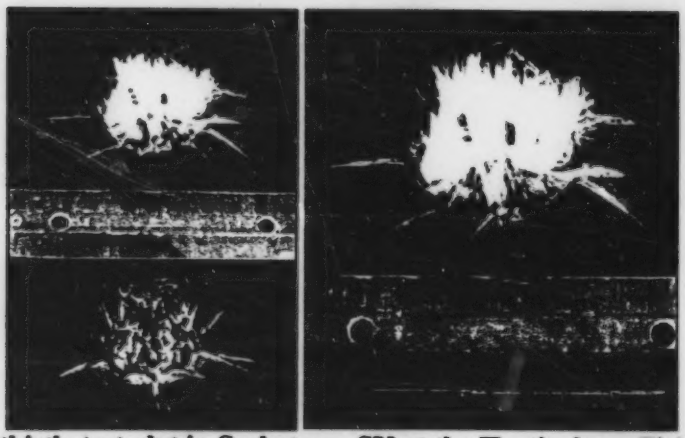


Fig. 3.31 Milk thistle test plot in Saskatoon, SK at the Horticulture Field Lab, 2001.

Fig. 3.32 Milk thistle seed head at second stage of maturity.

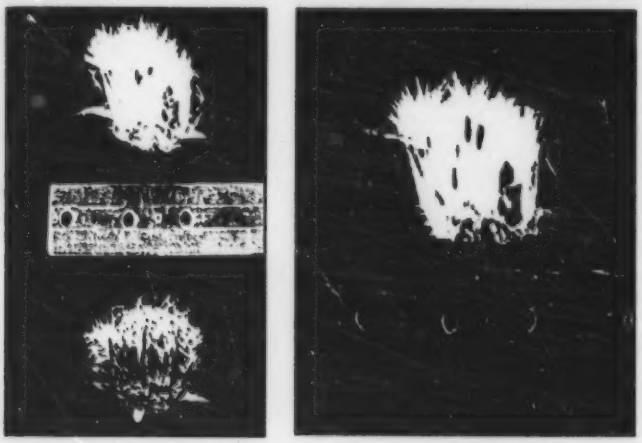


Fig. 3.33 Mature milk thistle seed head and seed.

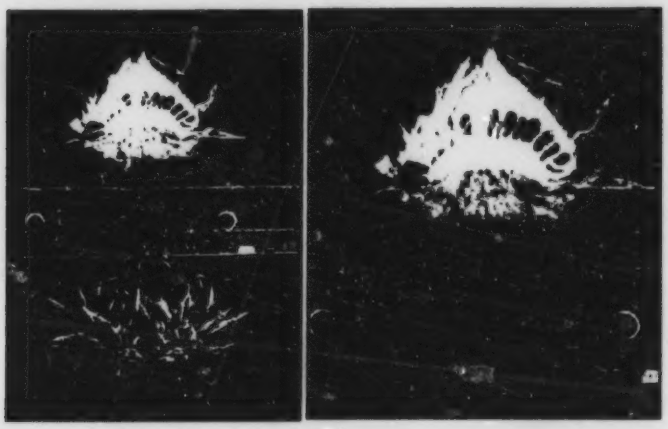


Fig. 3.34 Milk thistle seed held at first stage of maturity.

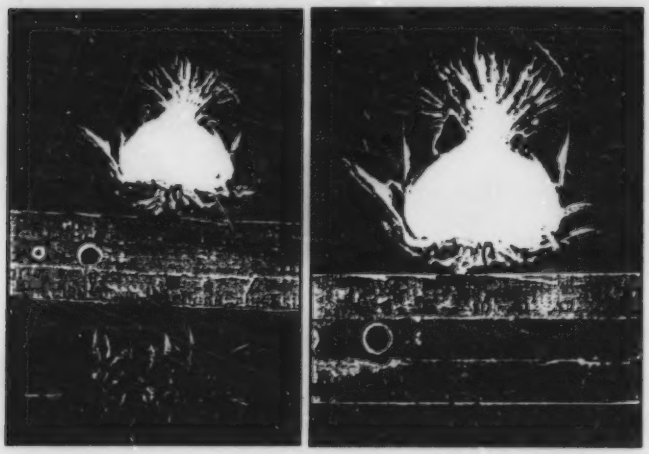


Fig. 3.35 Immature milk thistle seed head.

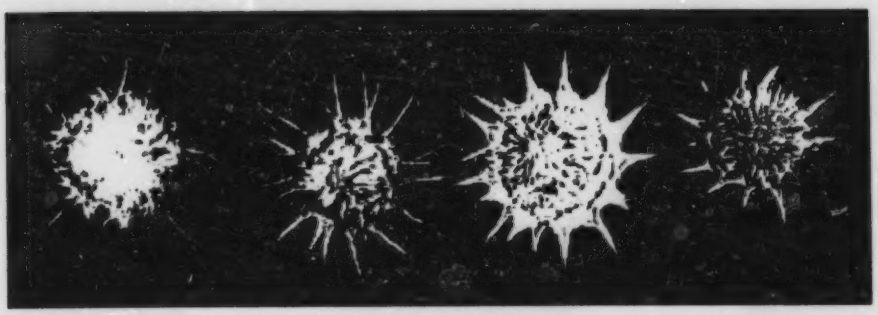


Fig. 3.36 Overview of all four stages of milk thistle head development. From left to right – Mature, Second stage maturity, first stage maturity and immature.

Fig. 3.37 Young milk thistle plant in Saskatoon, SK research plot on June 30th 2001.

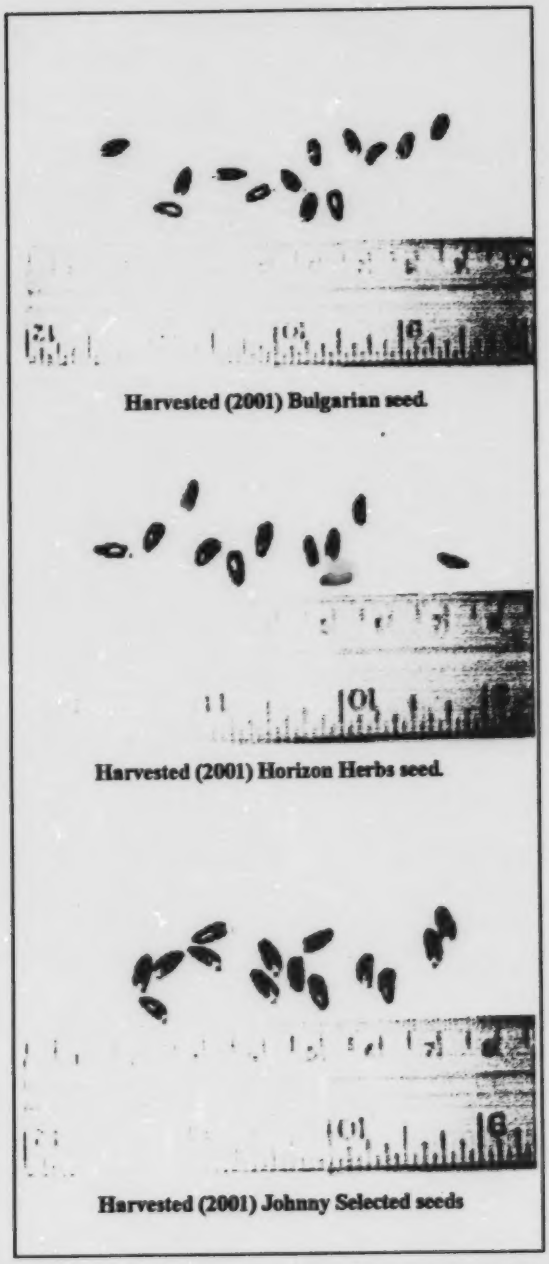


Fig. 3.38 Milk thistle seeds harvested from the 2001 test plots.

Table 3.32 Comparison of seed sources and date of harvest in terms of seed yield (g) per plant 2001.

Seed Source	Date of Harvest	Seed Yield (g) Per Plant
Bulgaria	September 5	23.78
	September 12	14.81
	September 19	32.57
	September 26	44.46
	October 4	24.76
Horizon Herbs, Oregon USA	September 5	4.28
	September 12	4.59
	September 19	9.60
	September 26	26.55
	October 4	13.63
Johnny's Selected Seeds, Maine USA	September 5	4.79
	September 12	6.48
	September 19	9.27
	September 26	29.76
	October 4	14.22
Statistical Significance		
Source		**
Harvest Date		**
Source*Harvest Date		ns
LSD		6.55
Source		7.74
Harvest Date		

† ** denotes $p \leq 0.01$; ns = not significant

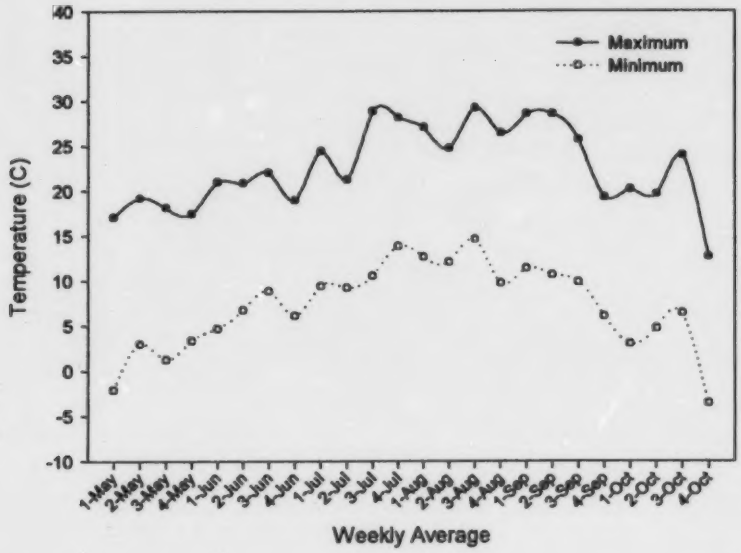


Fig 3.39 Weekly maximum and minimum average temperatures (°C) for Saskatoon, SK During the months of May to October in 2001.

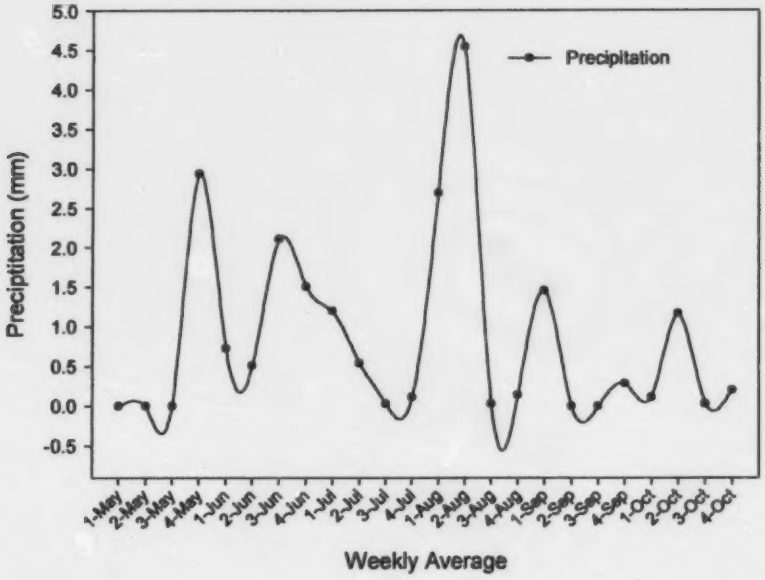


Fig. 3.40 Average weekly precipitation (mm) for the months of May to October for Saskatoon, SK in 2001.

From: Wheaton, E. (2001). Changing Climates: Exploring Possible Future Climates of the Canadian Prairie Provinces. Saskatchewan Research Council, Saskatoon, SK.

Table 3.33 Ranges of average temperature changes (EC) across the Prairies for the 2020s, 2050s and 2080s time slices as compared to the 1961-90 simulation. Results are from the Canadian Global Climate Model forced with greenhouse gases only [CGCM1GG] (CCIS 2000).

Season	Time Slice		
	2020s (2010-2039)	**2050s** (2040-2069)	**2080s** (2070-2099)
Annual	2 to 3	4 to 5	5 to 8
Spring	2 to 3	4 to 7	5 to 9
Summer	2 to 3	3 to 5	5 to 7
Fall	1 to 2	2 to 4	3 to 5
Winter	2 to 4	5 to 8	5 to 9

The main characteristics of these average temperature changes through these time and space scales include:

I. Considerable increases with time, especially for the last period, the 2080s.
II. Largest increases for the winter, then spring, and smallest increase for the fall.
III. Greatest spatial variation for the winter, then for the spring and least for the fall.

3.8 *Tanacetum parthenium* (Feverfew)

The objective of this study was to determine if there was a notable difference in the biomass and parthenolides between three seed sources: Horizon Herbs, Oregon; Johnny's Selected Seeds, Maine; and Richter's, Ontario. All plants were harvested at the same stage of development.

3.8.1 Germination

Germination of seed was within a 25°C greenhouse. Seedlings were fertilized weekly with a five hundred (500g/L) solution of 20:20:20 (N:P:K:). Seedlings were hardened off under a shade house and then planted at the Horticulture Field Lab in Saskatoon. Soil moisture levels were monitored as was deemed necessary.

3.8.2 Transplant date and date of first flower

Plants produced from Richter's (Ontario) had a shorter time to first flower compared to Horizon Herbs (Oregon) and Johnny's Selected Seeds (Maine) (Table 3.34). While this was likely due to a 2-week advanced planting date, it resulted in a 3 week advancement in flowering.

3.8.3 Harvest

Since planting dates varied, time to reach developmental stages were different between Richter's Seed source plants and the other 2 sources. Therefore, harvest dates for the various stages also varied (Table 3.35). Johnny's Selected Seeds (Maine) and Horizon Herbs (Oregon) both had higher upper plant biomass compared to Richter's.

3.8.4 Yield and Future Prospect

While upper plant biomass was greater for both Johnny's Selected Seeds and Horizon Herbs compared to Richter's, Johnny's Selected Seeds also had roughly double the floral biomass compared to the other 2 seed sources (Table 3.40). Transplant dates were quite late (June 14 – 29) and should be repeated with earlier transplant dates and common harvest dates. The 2001-year was warm and relatively dry (Figs. 3.41 and 3.42) and may be more typical of the projected climate in the prairies to 2080 (Table 3.33). Even with late planting, harvest still occurred during the first week in September and therefore, this crop should normally mature in time to avoid fall frosts. Potential differences between sites are apparent but require further testing before definitive conclusions can be made.

Table 3.34 *Tanacetum parthenium* (Feverfew) transplanting, flowering and harvesting dates for *Tanacetum parthenium* in Saskatoon, SK. in 2001.

Year	Seed Source	Transplant Date	Date of First Flower
2001	*Johnny's Selected Seeds, Maine*	June 29th	August 20th
	Horizon Herbs, Oregon	June 29th	August 22nd
	Richter's, Ontario	June 14th	August 1st

Table 3.35 *Tanacetum parthenium* (Feverfew) harvest dates of various plant parts for the three seed sources.

Plant Part	Seed Source	Harvest Date	Mean Dry Weight (g) Per Half of a Single Plant
Pre-flowering Leaves	Richter's, Ontario	Not Harvested	-
	Johnny's Selected Seeds, Maine	August 10	7.36
	Horizon Herbs, Oregon	August 13	13.63
Top Half	Richter's, Ontario	August 10	24.26
	Johnny's Selected Seeds, Maine	September 5	55.53
	Horizon Herbs, Oregon	September 7	44.56
Leaves	Richter's, Ontario	August 10	5.13
	Johnny's Selected Seeds, Maine	September 5	7.30
	Horizon Herbs, Oregon	September 7	8.10
Flowers	Richter's, Ontario	August 10	5.00
	Johnny's Selected Seeds, Maine	September 5	11.93
	Horizon Herbs, Oregon	September 7	6.87

3.9 *Angelica sinensis* (Chinese Angelica/Dong-quai)

3.9.1 Germination

Angelica seeds were scattered on top of a seeding tray containing Sunshine Mix #5 and placed in a misting chamber with bottom heat. Only 5% germination occurred with sequential plantings. Seedlings were then ordered from Richter's and were to be delivered to all six sites in June of 1999. This order has been cancelled due to supply problems and therefore the angelica plots could not be planted for this season. In 2000, however, seedlings did not establish well and crops failed before first flowering (Table 3.36, Figs. 3.40, 3.41).

Table 3.36 *Angelica polymorpha* var. sinensis transplanting and harvesting dates for all sites in 2000.

Year	Site	Transplant Date	Date of First Flowering	Date of First Harvest
2000	*Madras, OR, USA*	Unknown	Crop Failure	Crop Failure
	Davis, CA, USA	Died before transplanting	Crop Failure	Crop Failure
	Saskatoon, SK, Canada	June 5th	Crop Failure	Crop Failure
	Outlook, SK, Canada	June 7th	Crop Failure	Crop Failure
	La Ronge, SK, Canada	June 5th	Crop Failure	Crop Failure

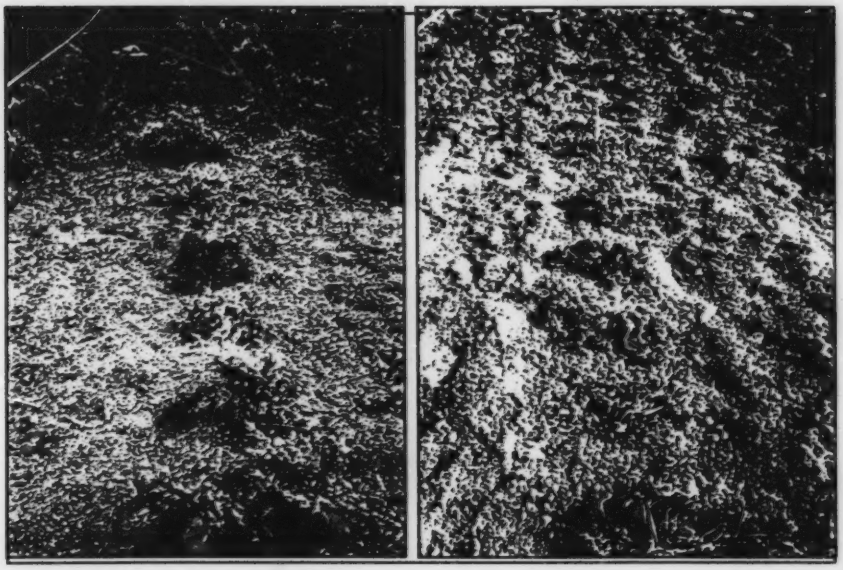


Fig. 3.41 *Angelica sinensis* in La Ronge, Saskatchewan during mid-August (left) and late September (right).

Fig. 3.42 *Angelica sinensis* in Saskatoon, Saskatchewan on August, 3rd 2000 showing signs of *Colletotrichum*.

All Saskatchewan sites were lost due to disease. Dr. Jill Thomson, Plant Pathologist for the department of Plant Sciences, analyzed samples of the *Angelica*. Vascular discoloring was found within the crown region as well as poor root development. When isolated, the samples were indicative of a *Colletotrichum* type, however, further testing would have been required for species determination. La Ronge had good stand establishment until mid-August, when the pathogen affected the entire plot (Fig. 3.27). By late September all of the plants were lost. Plant material was also lost in Saskatoon and Outlook.

As per personal communication with Dr. Peter Sexton's technician, Rhonda Bafus in Oregon, the *Angelica* was started before April 5th and was placed at the recommended 4°C germination temperature. They had difficulty maintaining this temperature with 70°F (20°C) weather at that time. On the 28th of April, the angelica still had not germinated and on our suggestion, she removed them from the cooler to the greenhouse. On the 9th of May, it was reported that the *Angelica* was healthy, however, on the 27th of June, she mentioned that the growth was quite slow. The *Angelica* plants never established well in the greenhouse as transplants. Once the transplants were moved outside to harden off, the intense heat killed all of the plant material. Plants were unable to be re-established again due to the lateness of the season.

A brief message was received from Herb Phillips at the University of California, Davis on the 5th of May stating that they too had difficulty with *Angelica* germination until the trays were removed from the cool temperatures to the greenhouse for germination. On the 2nd of June, the *Angelica* was said to be quite weak and would need more time before transplanting would be able to occur. In June the *Angelica* did not survive the lath house hardening off after the location had experienced +42°C temperatures accompanied by 64 km/h winds. Due to the lateness of the season, it was not possible to produce new seedlings in time for re-planting.

4.0 PERSONNEL

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Dr. Branka Barl

Kristine Krieger, Research Assistant
Sandy Maybin, Technician
Arin Katrusiak, Research Assistant

Co-operators:

Gerry Ivanochko - Saskatchewan Agriculture and Food, La Ronge, SK
Bill & Jean King – Outlook, SK
Dr. Peter Sexton – Oregon State University, Madras, Oregon
Rhonda Bafus – Technician, Oregon State University, Madras, Oregon
Dr. Jim Simon – Purdue University, West Lafayette, Indiana
Dr. Ron Voss – UC Davis, Davis, California
Herb Phillips – US Davis, Davis California

5.0 EQUIPMENT:

Water Pump Engine

6.0 ACKNOWLEDGEMENTS:

None

Section B) PHYTOCHEMICAL STUDIES AND QUALITY ANALYSIS

8.0 Introduction

The overall objective of this section of the project is to test for Northern Vigor® response in selected medicinal and aromatic plants (echinacea, calendula, catnip, burdock and valerian) by probing for a combined latitudinal and environmental effect on phytomedicinal quality of plants over two growing seasons. In late fall and early winter of 1999 and 2000, Herb Research Program (HRP) was provided with samples from La Ronge, Saskatoon and Outlook in Saskatchewan (51.3°N to 55.1°N) by Tanino and Krieger, from Madras, Oregon (44.6°N), by Peter Sexton from Central Oregon Agriculture Research Station affiliated with Oregon State University in Corvallis, and from Davis, California (38.5°N), by Herb Phillips from University of California at Davis. Samples of all plants from all sites, but echinacea from Davis were provided in time for testing. In 2001, also included in this study were echinacea (third growing season), and feverfew and milk thistle (first growing season) in Saskatchewan only.

9.0 *Echinacea angustifolia* (Narrow-leaved Purple Cone-flower)

Echinacea is used medicinally for the following therapeutic indications: prevent or decrease symptoms of upper respiratory tract infections, such as colds and flu. Many chemical constituents have been reported in the literature, but phenolic compounds (particularly echinacoside for *E. angustifolia* and *E. pallida*), alkylamides and polysaccharides are most frequently mentioned as markers for quality determination of echinacea root.

Objectives

1. to determine content of phenolic markers (echinacoside, cynarin, chlorogenic acid) in echinacea root by HPLC.
2. to determine root alkylamide profile and relative content by HPLC.

Materials and Methods

Plant Material

In 1999, sixty-eight (68) samples of echinacea dry root harvested October 15-20 in Saskatchewan and November 15 in Madras, Oregon, were provided to the HRP for testing. Two samples per replicate were pooled to provide 8 samples of healthy one-year root for analysis for each site in Saskatchewan. Four samples each of echinacea root grown under standard conditions and using soil supplementation with sulfur in Madras, Oregon, were also analyzed. Echinacea from Davis, California, was not provided due to crop failure. Up to 5% of echinacea plants at all 3 Saskatchewan sites showed symptoms of aster yellows disease. A total of 12 samples, 4 samples from each Saskatchewan site, were analyzed for the effect of aster yellows infection on phytomedicinal quality.

In 2000, thirty-two (32) samples of echinacea dry root, 2 plant samples per row for each of 4 rows, harvested October 19-23 in Saskatchewan and November 3 in Madras were provided to the HRP for testing. These were one-year old root samples, since echinacea needed to be

replanted at all sites in 2000 as elaborated in the report for the agronomic part of the study provided by Tanino and Krieger. Echinacea from Davis was not provided due to crop failure.

In 2001, thirty-two (32) samples of two-year echinacea dry root were provided, 4 plant samples per row for each of four rows, harvested on October 16, 2001 in Saskatoon and Outlook.

Sample Preparation

Each sample consisting of the entire echinacea root was cleaned of residual dirt, ground in a hammer mill and passed through a 30 mesh sieve. A sub-sample of the resulting powder (1.0 g) was extracted with 100 mL methanol for 20 minutes under reflux. An aliquot of the extract was filtered through a cotton wool, centrifuged at 10,000 RPM for 5 min and an aliquot of the supernatant filtered through a 0.45 μm micro-filter prior to being analyzed for the content of phenolic markers and alkylamides by high performance liquid chromatography (HPLC).

Liquid Chromatography

An HPLC analysis of root extract for phenolic markers (echinacoside, cynarin and chlorogenic acid) and alkylamides was performed with Waters system using the Millennium 32 Chromatography Manager software and Waters Nova-Pak RP_{18} column (4μm, 3.9 x 150 mm). Phenolic markers were analyzed by gradient elution starting with 95 % B (water containing 1% of 0.1N H_3PO_4) : 5% A (acetonitrile containing 1% of 0.1N H_3PO_4) to 70 % B : 30% A over 25 min using flow rate of 1 mL/min and injection volume of 5 μL. Echinacoside, cynarin and chlorogenic acid were detected by PDA at λ=330 nm. Analysis time was 35 min. Seven-point calibration curve was generated for each reference phenolic compound in methanol in the range of 20–2000 ng. Standards were purchased from Analyticon, Germany and Sigma, U.S.A. Alkylamides were analyzed by gradient elution starting with 60 % B (water) : 40% A (acetonitrile) to 40 % B : 60% A over 30 min using flow rate of 1 mL/min and injection volume of 20 μL. They were detected by PDA at λ=210 nm and 254 nm; analysis time is 35 min. The retention time of seven main alkylamides ranged from 9.3 min to 23.8 min. Given that pure alkylamide standards were not commercially available, only relative content of each of 7 alkylamides expressed as peak area units per gram of root was determined.

High Performance Thin Layer Chromatography

HPTLC analysis of carbohydrates was performed using silica gel $60F_{254}$ (Merck) TLC plates. Sample was applied using a Linomat IV (Camag) chromatography system. Solvent system of n-butanol:acetic acid:water was used as a developing solvent. Plates were scanned using a Hitachi high resolution CCD camera and data were processed digitally with Camag chromatography software and Corel Photo-Paint.™

Results

The HPLC profile of selected phenolic compounds (echinacoside, cynarin and chlorogenic acid) and HPLC profile of alkylamides in *Echinacea angustifolia* root are presented in Fig. 1 and 2, respectively. The five main alkylamide peaks with retention times of 9.3 min, 10.9 min, 12.0 min, 15. 9 min and 18.8 min accounted for above 80% of all alkylamides present in the root.

In 1999, the content of three target phenolic compounds, echinacoside, cynarin and chlorogenic acid, in one-year old root samples from 3 Saskatchewan sites and a single US site, Madras, Oregon, is presented in Table 1. The content of echinacoside, a dominant phenolic compound, ranged from 0.44% (Madras) to 1.66% (Saskatoon), and was significantly higher in all Saskatchewan grown roots than in US grown roots. Crop failed in Davis, so no data for that site could be provided. With respect to the alkylamide levels, echinacea samples from all sites were comparable (Table 2).

A preliminary analysis of carbohydrate profile of an aqueous root extract of 1999 crop (Fig. 3) showed a presence of a great range of carbohydrate molecules with respect to molecular weight (MW), from monosaccharides (fructose) to disaccharides (sucrose) and high MW compounds. However, a general profile appeared consistent in all samples and was not influenced by the production site. More in-depth analysis of carbohydrate composition using more sensitive assay would be desirable to probe the potential link between the environmental factors, such as latitude, and polysaccharide composition. This is particularly relevant given the suspected role of high MW polysaccharides from echinacea in enhancing the immune responses in animals and humans. Sucrose appeared to be one of dominant compounds in all samples tested, and seem to increase in aster yellows infected plants.

The first year data strongly suggest that *E. angustifolia* grown at higher latitudes (for example in Saskatchewan vs. California), accumulated significantly higher amounts of target phenolic compounds, which may or may not be related to the immunostimulatory activity of echinacea.

In 2000, the content of three phenolic compounds and alkylamides in one-year old root samples from three Saskatchewan sites and a single US site, Madras, is presented in Table 3 and 4, respectively. The content of echinacoside ranged from 0.66% (Madras) to 1.62% (Saskatoon), and was significantly higher in all Saskatchewan samples than in US samples. Crop failed in Davis again, so no data for that site could be provided. The second year data strongly suggested that *E. angustifolia* grown at higher latitudes accumulated significantly higher amounts of echinacoside, a dominant phenolic marker used for quality grading of echinacea root. It was on average twice as high across Saskatchewan sites (1.36%) in comparison to Oregon site. Results are comparable to the results obtained in the first growing season, 1999.

Relative content and composition of alkylamides in one-year old root (Table 5) was significantly higher, on avearge 61% higher, in all Saskatchewan sites than in Madras; mean value for total alkylamides in area units was 27 $x10^9$ for Saskatchewan as compared to 17 $x10^9$ for Madras. The main alkylamide compound, peak with retention time 15.9 min known from literature to be dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide, made about 30% of total alkylamides in all echinacea root samples tested. It was also on average 56% higher in Saskatchewan- than in Oregon-grown root. These results provide further evidence for a latitudinal and environmental effects on phytomedicinal quality of *Echinacea angustifolia* root suggesting indeed an expression of Northern Vigor® response.

In 2001, the content of target phenolic compounds and alkylamides in two-year old root samples from two Saskatchewan sites, Saskatoon and Outlook, is presented in Table 5 and 6,

respectively. On average, levels of both classes of compounds, phenolics and alkylamides, were lower in two-year old root than in one-year old root from a same site. Echinacoside content of two-year root was 0.97% vs. 1.40% of one-year root in Saskatoon, and 0.89% vs. 1.05% in Outlook. Similar trends were also observed with other two phenolic compounds, cynarin and chlorogenic acid. Data for seven alkylamide compounds further indicate a decreasing trend in bioactive molecules in two-year old roots in comparison to one-yea old roots. In former studies of the HRP it was found that two-year roots had on average higher content of bioactive molecules than three-year old roots.

Soil supplementation with sulfur caused a slight increase in the content of phenolics, as suggested by the analysis of Madras root samples in 1999 (Fig. 4). Relative content and composition of alkylamides in one-year old root was comparable across all sites (Fig. 5). The relative content of seven compounds with retention times 9.3 min to 23.7 min (their chemical structures are known from the literature, but pure compounds were not available commercially, thus absolute contents could not be determined) were determined as a ratio of a single peak area to the total peak area. Sulfur had no affect on the alkylamide content. Larger number of samples from more than one production site need to be analyzed before any recommendation for soil sulfur content in echinacea production could be provided.

The effect of aster yellows disease on echinacea root quality was probed in 1999. The disease caused decrease in the content of phenolics in root samples from La Ronge and Saskatoon, but an increase in samples from Outlook (Fig. 6). Similar trends across Saskatchewan sites was observed for alkylamides levels (Fig. 7). No conclusive evidence for anticipated negative effect of aster yellows on root quality could be made at present.

As a general conclusion, a strong evidence for the latitudinal and environmental effects on phytomedicinal quality of echinacea was provided in this study. This means that Saskatchewan has a competitive advantage in producing premium quality echinacea root in North America. With respect to the economics of echinacea root production, however, further investigation into the optimal production cycle (one year, two-year and three year) with respect to both yield and quality for echinacea crop is required.

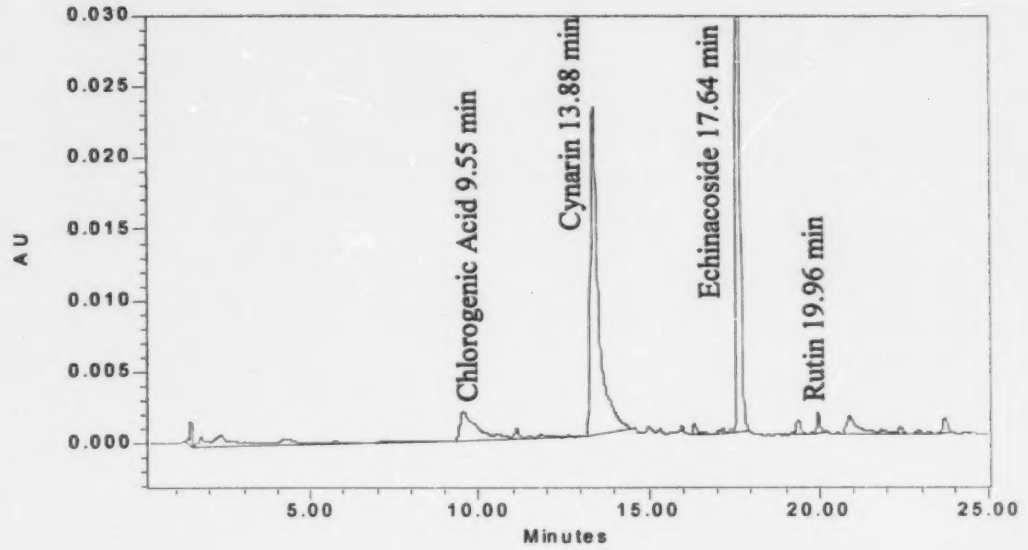


Figure 1. HPLC profile of selected phenolic compounds in *Echinacea angustifolia* root.

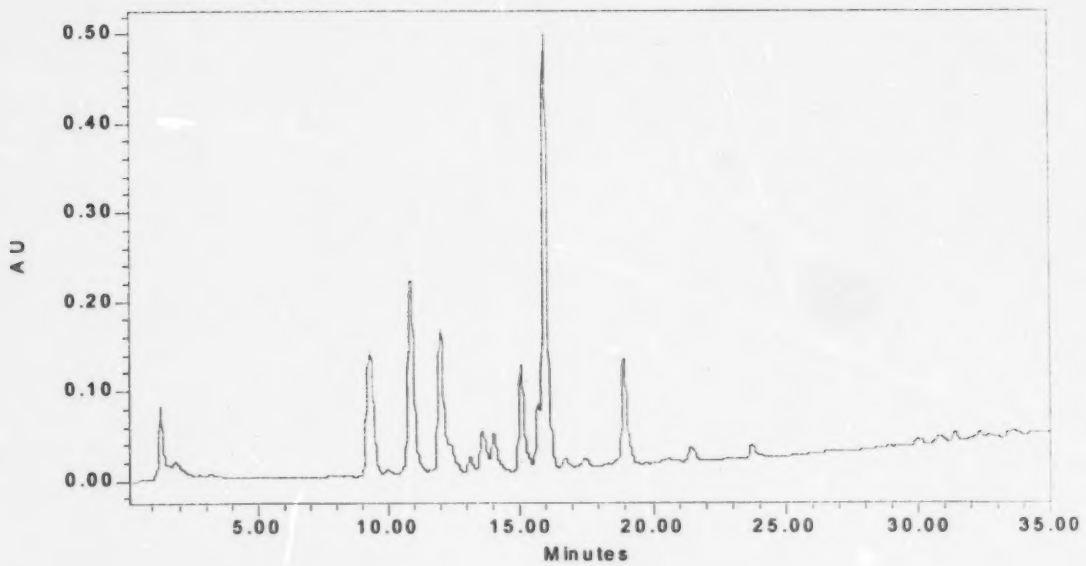


Figure 2. HPLC profile of alkylamides in *Echinacea angustifolia* root.
(Peaks with RT = 9.3; 10.9; 12.0; 15. 9 and 18.8 min have been quantified).

Table 1. Content of Phenolic Marker Compounds in One-Year *E. angustifolia* Root[1] Harvested in Fall 1999.

Sample ID	Moisture Content (%)	Echinacoside (%)[2] Mean±S.D.	Cynarin (%)[2] Mean±S.D.	Chlorogenic Acid (%)[2] Mean±S.D.
La Ronge, SK				
R1-1	12.52	1.87	0.21	0.16
R1-2	13.80	1.09	0.39	0.16
R2-1	12.22	2.34	0.42	0.19
R2-2	13.24	1.30	0.21	0.15
R3-1	15.48	1.68	0.27	0.14
R3-2	13.89	0.92	0.25	0.19
R4-1	11.23	1.17	0.32	0.24
R4-2	14.75	0.99	0.40	0.26
Mean±S.D.		**1.42±0.50**	**0.31±0.09**	**0.19±0.04**
Outlook, SK				
R1-1	14.67	1.05	0.16	0.10
R1-2	10.80	1.00	0.23	0.18
R2-1	11.47	0.87	0.25	0.10
R2-2	9.82	0.53	0.12	0.06
R3-1	11.55	0.85	0.32	0.18
R3-2	11.32	1.17	0.25	0.12
R4-1	11.95	1.24	0.17	0.09
R4-2	12.41	0.54	0.12	0.06
Mean±S.D.		**0.91±0.27**	**0.20±0.07**	**0.11±0.04**
Saskatoon, SK				
R1-1	11.03	1.50	0.14	0.26
R1-2	13.02	0.96	0.25	0.15
R2-1	11.56	1.53	0.29	0.19
R2-2	11.17	1.37	0.18	0.17
R3-1	10.69	2.46	0.36	0.29
R3-2	10.44	2.62	0.42	0.26
R4-1	12.53	1.19	0.28	0.11
R4-2	12.28	1.63	0.23	0.09
Mean±S.D.		**1.66±0.58**	**0.27±0.09**	**0.19±0.07**
Madras, OR				
R1-1	13.63	0.85	0.11	0.07
R2-1	14.72	0.32	0.06	0.04
R3-1	14.61	0.26	0.08	0.04
R4-1	15.38	0.33	0.07	0.05
Mean±S.D.		**0.44±0.27**	**0.08±0.02**	**0.05±0.01**

[1]One-year root (plants transplanted in 1999; root harvested Oct. 20 in Saskatoon, Oct. 18 in Outlook, Oct. 15 in La Ronge, Nov. 15 in Madras 1999)

[2]Content of echinacoside, cynarin and chlorogenic acid are reported on dry weight basis

Table 2. Alkylamides Content[1] of One-Year *E. angustifolia* Root Harvested in Fall 1999.

Sample ID	Area (areax109) RT=9.3 min	Area RT=10.9 min	Area RT=12.0 min	Area RT=15.9 min	Area RT=18.8 min	Total Area
Saskatoon, SK						
R1-1	1.89	1.74	1.10	3.00	1.89	9.94
R1-2	3.07	3.22	1.46	6.73	2.78	17.70
R2-1	1.88	2.23	1.37	5.65	3.08	14.72
R2-2	2.61	3.00	1.17	4.56	3.00	14.86
R3-1	2.01	1.86	1.43	2.87	1.15	9.75
R3-2	2.11	2.60	0.81	6.16	2.11	14.92
R4-1	3.12	3.43	2.19	6.87	4.06	22.17
R4-2	3.89	4.23	3.23	9.12	3.89	26.92
Analyte Mean ± S. D.	**2.57±0.73**	**2.79±0.85**	**1.60±0.77**	**5.62±2.10**	**2.75±0.99**	**16.37±5.84**
Outlook, SK						
R1-1	3.46	3.58	0.86	6.42	2.96	18.76
R1-2	1.74	2.18	1.16	5.09	2.03	12.21
R2-1	2.65	3.18	1.99	6.22	2.91	18.41
R2-2	2.62	3.37	1.37	4.49	2.50	14.35
R3-1	2.31	2.62	2.46	6.77	4.31	20.32
R3-2	2.76	2.37	2.10	6.84	4.34	19.20
R4-1	2.68	2.97	2.68	7.29	4.02	21.27
R4-2	2.16	2.42	2.03	4.96	4.20	17.30
Analyte Mean ± S. D.	**2.55±0.50**	**2.84±0.51**	**1.83±0.64**	**6.01±1.03**	**3.41±0.91**	**17.73±3.05**
La Ronge, SK						
R1-1	2.03	0.48	1.55	5.98	2.99	16.38
R1-2	2.94	3.40	2.94	8.51	4.49	25.23
R2-1	2.79	2.35	1.91	6.16	3.38	17.02
R2-2	2.54	2.05	1.09	6.64	4.59	17.38
R3-1	2.53	2.84	2.05	8.05	5.68	22.74
R3-2	1.56	1.74	0.69	4.52	1.56	10.08
R4-1	2.74	2.88	1.78	6.72	3.98	21.66
R4-2	3.30	3.66	1.47	8.24	4.58	22.53
Analyte Mean ± S. D.	**2.55±0.54**	**2.43±1.02**	**1.69±0.67**	**6.85±1.36**	**3.91±1.26**	**19.13±4.86**
Madras, OR						
R1-1	1.19	1.34	0.59	2.38	0.74	6.68
R1-2	2.44	2.79	3.72	7.32	2.21	20.32
R2-1	2.73	3.02	1.98	5.66	2.54	16.97
R2-2	2.59	2.73	1.64	5.59	2.04	16.08
R3-1	3.49	2.89	2.49	6.18	3.49	22.03
R3-2	2.17	2.07	2.56	4.63	1.58	14.08
R4-1	2.17	1.85	1.30	5.98	1.85	14.03
R4-2	2.44	2.95	2.74	8.54	3.86	22.77
Analyte Mean ± S. D.	**2.40±0.64**	**2.45±0.62**	**2.13±0.96**	**5.78±1.82**	**2.29±1.01**	**16.62±5.25**

[1]Relative alkylamides content expressed as peak area x 10^9 units (λ = 210 nm) per gram of root; total area is sum of 5 peaks plus minor peaks

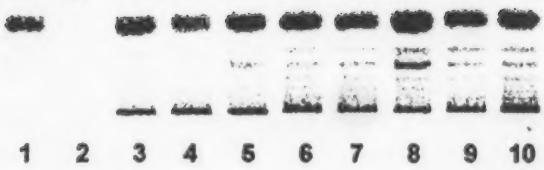


Figure 3. TLC profile of carbohydrates in *Echinacea angustifolia* root.

List of TLC lanes:

1. Sucrose reference
2. D (-) Fructose reference
3. Oregon sample #1
4. Oregon sample #2
5. La Ronge sample
6. Outlook sample
7. Saskatoon sample
8. La Ronge sample of diseased root
9. Outlook sample of diseased root
10. Saskatoon sample of diseased root

Table 3. Content of Phenolic Marker Compounds in One-Year *E. angustifolia* Root[1] Harvested in Fall 2000.

Sample ID	Moisture Content (%)	Echinacoside[2] (%)	Cynarin[2] (%)	Chlorogenic Acid[2] (%)
La Ronge, SK				
R1-1	11.42	1.69	0.37	0.18
R1-2	10.67	1.14	0.63	0.24
R2-1	9.51	1.66	0.35	0.18
R2-2	8.65	1.55	0.34	0.23
R3-1	8.61	1.65	0.56	0.18
R3-2	8.42	2.21	0.53	0.22
R4-1	11.22	1.93	0.66	0.42
R4-2	10.62	1.12	0.41	0.15
Mean±S.D.[3]		**1.62±0.37**	**0.48±0.13**	**0.23±0.08**
Outlook, SK				
R1-1	10.09	1.27	0.46	0.14
R1-2	9.72	0.86	0.53	0.15
R2-1	7.92	0.93	0.54	0.17
R2-2	9.79	0.85	0.44	0.14
R3-1	8.82	1.13	0.52	nd[4]
R3-2	9.43	1.14	0.27	nd
R4-1	9.66	1.46	0.49	0.16
R4-2	7.56	0.76	0.43	0.14
Mean±S.D.		**1.05±0.24**	**0.46±0.09**	**0.11±0.07**
Saskatoon, SK				
R1-1	8.70	1.27	0.45	0.12
R1-2	9.14	1.41	0.35	0.18
R2-1	9.23	2.12	0.72	0.39
R2-2	9.40	1.34	0.37	0.21
R3-1	10.68	1.95	0.53	0.18
R3-2	9.05	1.37	0.73	0.13
R4-1	8.50	0.92	0.36	0.14
R4-2	9.66	0.83	0.51	0.16
Mean±S.D.		**1.40±0.45**	**0.50±0.15**	**0.19±0.08**
Madras, OR				
R1-1	7.81	0.53	0.16	0.14
R1-2	10.09	0.74	0.32	0.14
R2-1	8.24	0.55	0.23	nd
R2-2	7.64	0.78	0.15	0.14
R3-1	8.77	0.64	0.26	0.12
R3-2	8.16	0.89	0.21	0.13
R4-1	9.02	0.76	0.18	nd
R4-2	9.42	0.40	0.14	0.17
Mean±S.D.		**0.66±0.16**	**0.21±0.06**	**0.11±0.07**

[1]One-year root (plants transplanted June 5, 2000; root harvested Oct. 19 in Saskatoon, Oct. 20 in Outlook, Oct. 23 in La Ronge, Nov. 3 in Madras 2000)

[2]Content of echinacoside, cynarin and chlorogenic acid are reported on dry weight basis

[3]Mean value for each analyte ± standard deviation is bolded

[4]nd-not detected

Table 4. Alkylamides Content[1] of One-Year *E. angustifolia* Root Harvested in Fall 2000.

Sample ID	Area RT=9.3 min (areax10⁹)	Area RT=10.9 min	Area RT=12.0 min	Area RT=15.9 min	Area RT=18.8 min	Total Area
Saskatoon, SK						
R1-1	4.79	4.97	3.40	8.08	5.12	31.38
R1-2	2.11	2.46	1.63	5.86	3.52	19.14
R2-1	4.16	5.20	3.59	9.27	5.34	32.31
R2-2	3.43	5.10	2.12	9.69	4.20	28.93
R3-1	2.96	4.24	3.42	8.31	5.67	30.04
R3-2	5.26	4.76	2.11	5.49	4.31	25.99
R4-1	5.50	5.66	1.65	10.89	5.83	36.46
R4-2	3.83	4.31	3.63	11.03	5.93	34.74
Mean ± S.D.	**4.00 ± 1.16**	**4.59 ± 0.98**	**2.69 ± 0.89**	**8.58 ± 2.08**	**4.99 ± 0.88**	**29.87 ± 5.43**
Outlook, SK						
R1-1	3.16	3.56	3.02	7.97	5.37	29.75
R1-2	5.59	6.35	4.89	10.94	5.35	38.83
R2-1	2.98	3.32	3.23	9.03	4.98	27.74
R2-2	4.09	4.19	3.05	7.73	5.38	35.55
R3-1	2.68	3.06	2.35	3.08	2.40	16.85
R3-2	7.50	5.64	3.50	13.90	6.68	48.11
R4-1	4.01	4.70	0.00	7.66	3.82	24.77
R4-2	2.68	3.64	2.91	5.86	3.15	21.60
Mean ± S.D.	**4.09 ±1.69**	**4.31 ± 1.18**	**2.87 ± 1.28**	**8.27 ± 3.23**	**4.64 ± 1.40**	**30.40 ± 10.08**
La Ronge, SK						
R1-1	2.91	2.95	2.30	7.33	3.85	26.39
R1-2	2.04	2.54	2.06	5.53	3.01	18.11
R2-1	2.47	2.38	2.62	5.79	4.79	21.91
R2-2	3.08	3.58	0.00	4.98	2.47	15.81
R3-1	2.92	3.25	2.02	7.62	3.73	22.97
R3-2	2.46	2.77	1.33	5.57	2.98	19.24
R4-1	3.13	3.64	0.00	5.52	3.47	20.72
R4-2	4.35	4.40	2.83	8.56	5.17	31.14
Mean ± S.D.	**2.92 ± 0.69**	**3.19 ± 0.67**	**1.65 ± 1.11**	**6.36 ± 1.29**	**3.68 ± 0.92**	**22.04 ± 4.88**
Madras, OR						
R1-1	3.08	3.03	2.48	8.55	2.75	22.22
R1-2	1.99	2.18	1.63	4.12	2.13	15.37
R2-1	3.00	2.54	3.67	8.67	5.95	29.23
R2-2	1.96	2.25	1.67	5.56	2.15	16.85
R3-1	2.41	2.57	1.03	4.26	2.69	17.02
R3-2	1.75	1.53	1.13	3.42	2.13	12.85
R4-1	3.92	3.81	0.00	2.23	2.01	15.23
R4-2	1.33	1.49	0.00	2.96	0.95	7.63
Mean ± S.D.	**2.43 ± 0.85**	**2.43 ± 0.76**	**1.45 ± 1.23**	**4.97 ± 2.45**	**2.59 ± 1.46**	**17.05 ± 6.41**

[1]Relative content of each of five alkylamides expressed as peak area x 10^9 units (λ = 210 nm) per gram of root; total area is sum of 5 peaks plus minor peaks

Table 5. Content of Phenolic Marker Compounds of Two-Year *E. angustifolia* Root[1] Harvested in 2001 in Saskatchewan.

Sample ID	Dry Root Weight (g)	Echinacoside[2] (%)	Cynarin[2] (%)	Chlorogenic Acid[2] (%)
Saskatoon				
R1-1	24.8	0.94	0.39	0.07
R1-2	32.1	1.22	0.23	0.04
R1-3	16.4	1.52	0.47	0.10
R1-4	19.3	0.58	0.19	0.05
R2-1	16.0	0.75	0.21	0.04
R2-2	49.0	0.37	0.19	0.03
R2-3	49.9	0.63	0.16	0.04
R2-4	19.6	0.86	0.37	0.09
R3-1	6.5	0.70	0.14	0.06
R3-2	15.0	1.24	0.22	0.04
R3-3	19.3	0.89	0.17	0.04
R3-4	11.2	0.99	0.26	0.07
R4-1	8.5	0.94	0.13	0.03
R4-2	34.3	0.53	0.22	0.05
R4-3	46.5	1.38	0.26	0.03
R4-4	10.9	1.95	0.26	0.05
Mean±S.D.[3]		**0.97±0.41**	**0.24±0.10**	**0.05±0.02**
Outlook				
R1-1	35.4; 31.8	0.58	0.20	0.05
R1-2	31.1; 23.1	0.87	0.11	0.04
R1-3	22.5; 17.4; 12.3	0.70	0.17	0.04
R1-4	37.6; 42.3	1.01	0.20	0.03
R2-1	20.8; 23.5	0.70	0.27	0.04
R2-2	77.2; 62.4	0.80	0.30	0.07
R2-3	20.3; 12.6	0.81	0.26	0.05
R2-4	30.3; 24.9	0.84	0.21	0.03
R3-1	27.7; 22.6	0.71	0.21	0.03
R3-2	77.9; 54.8	1.05	0.23	0.04
R3-3	19.5; 16.5	1.12	0.25	0.04
R3-4	27.8; 37.8	0.55	0.18	0.04
R4-1	34.9	1.46	0.34	0.08
R4-2	27.9; 22.0	0.76	0.11	0.04
R4-3	60.5; 50.5	0.92	0.22	0.04
R4-4	20.0; 21.7	1.36	0.21	0.06
Mean±S.D.		**0.89±0.26**	**0.22±0.06**	**0.04±0.01**

[1]Two- year root (plants transplanted June 5, 2000; root harvested Oct. 16, 2001)

[2]Content of echinacoside, cynarin and chlorogenic acid are reported on as is basis

[3]Mean value for each analyte ± standard deviation is bolded

Table 6. Alkylamides Content[1] of Two-Year *E. angustifolia* Root[2] Harvested in 2001 in Saskatchewan.

Sample ID	Dry Root Weight (g)	Area RT=9.4 min	Area RT=11.0 min	Area RT=12.1 min	Area RT=16.0 min	Area RT=19.0 min	Area RT=21.5 min	Area RT=23.8 min	Total area
Saskatoon									
R1-1	24.8	0.95	0.99	1.39	3.03	0.90	0.10	0.20	10.40
R1-2	32.1	1.25	1.35	1.85	3.69	1.50	0.20	0.25	13.19
R1-3	16.4	0.75	0.80	0.85	1.89	0.60	0.05	0.50	8.45
R1-4	19.3	0.75	1.09	1.69	6.32	0.70	0.20	0.15	14.42
R2-1	16.0	0.85	1.15	1.90	3.94	0.85	0.15	0.25	12.73
R2-2	49.0	1.74	1.84	2.04	3.64	1.69	0.15	0.20	15.90
R2-3	49.9	1.35	1.35	0.90	4.11	0.85	0.05	0.10	10.84
R2-4	19.6	2.24	2.54	2.44	4.38	1.39	0.15	0.35	17.92
R3-1	6.5	1.75	2.04	0.10	3.49	1.10	0.05	0.25	12.38
R3-2	15.0	0.90	0.95	1.35	1.79	0.25	0.05	0.15	9.48
R3-3	19.3	1.50	1.30	0.35	2.84	1.45	0.10	0.20	11.89
R3-4	11.2	0.80	1.00	0.80	1.65	0.75	0.00	0.10	7.56
R4-1	8.5	0.75	0.75	1.00	2.44	0.65	0.00	0.35	9.24
R4-2	34.3	0.65	0.80	1.15	3.24	1.25	0.15	0.25	11.04
R4-3	46.5	0.80	0.95	1.25	3.54	0.65	0.10	0.15	10.38
R4-4	10.9	4.42	4.97	3.38	6.61	1.94	0.15	0.65	27.82
Mean ± S.D.		**1.34±0.94**	**1.49±1.05**	**1.40±0.81**	**3.54±1.41**	**1.03±0.46**	**0.10±0.06**	**0.26±0.15**	**12.73±4.86**
Outlook									
R1-1	35.4; 31.8	1.65	1.60	1.35	3.89	1.75	0.15	0.20	13.93
R1-2	31.1; 23.1	1.74	1.69	1.34	3.13	1.09	0.10	0.25	13.19
R1-3	22.5; 17.4; 2.28	1.55	1.85	1.65	4.50	1.65	0.25	0.15	14.31
R1-4	37.6; 42.3	1.44	1.44	0.79	2.73	1.04	0.00	0.25	10.16
R2-1	20.8; 23.5	1.05	1.15	1.35	2.14	0.90	0.10	0.20	8.78
R2-2	77.2; 62.4	2.69	3.03	2.54	5.07	1.89	0.20	0.20	18.10
R2-3	20.3; 12.6	1.49	1.79	1.00	2.09	0.65	0.10	0.15	9.68
R2-4	30.3; 24.9	1.20	1.29	0.80	3.74	1.00	0.10	0.15	9.54
R3-1	27.7; 22.6	1.89	2.18	0.70	4.32	0.15	0.15	0.20	13.55
R3-2	77.9; 54.8	1.79	2.24	0.80	4.77	1.24	0.10	0.20	13.18
R3-3	19.5; 16.5	1.99	2.59	2.24	4.04	1.20	0.10	0.25	15.85
R3-4	27.8; 37.8	1.49	1.94	1.29	2.84	0.85	0.10	0.15	10.85
R4-1	34.9	2.79	3.69	2.94	4.63	1.35	0.15	0.25	19.72
R4-2	27.9; 22.0	3.39	3.19	3.74	6.23	2.59	0.45	0.35	25.89
R4-3	60.5; 50.5	2.74	3.04	2.44	6.18	1.74	0.15	0.30	20.31
R4-4	20.0; 21.7	2.28	2.28	1.44	5.22	1.84	0.10	0.50	16.76
Mean ± S.D.		**1.95±0.66**	**2.19±0.74**	**1.65±0.89**	**4.09±1.28**	**1.31±0.59**	**0.14±0.10**	**0.23±0.09**	**14.61±4.68**

[1]Relative alkylamides content expressed as peak area x 10^9 units (λ = 210 nm) per gram of root; total area is sum of 7 peaks plus minor peaks

[2] Two- year root (plants transplanted June 5, 2000; root harvested Oct. 16, 2001)

Figure 4. Effect of Sulfur Treatment on Phenolic Marker Content of One-Year *E. angustifolia* Root Grown at Madras, 1999.

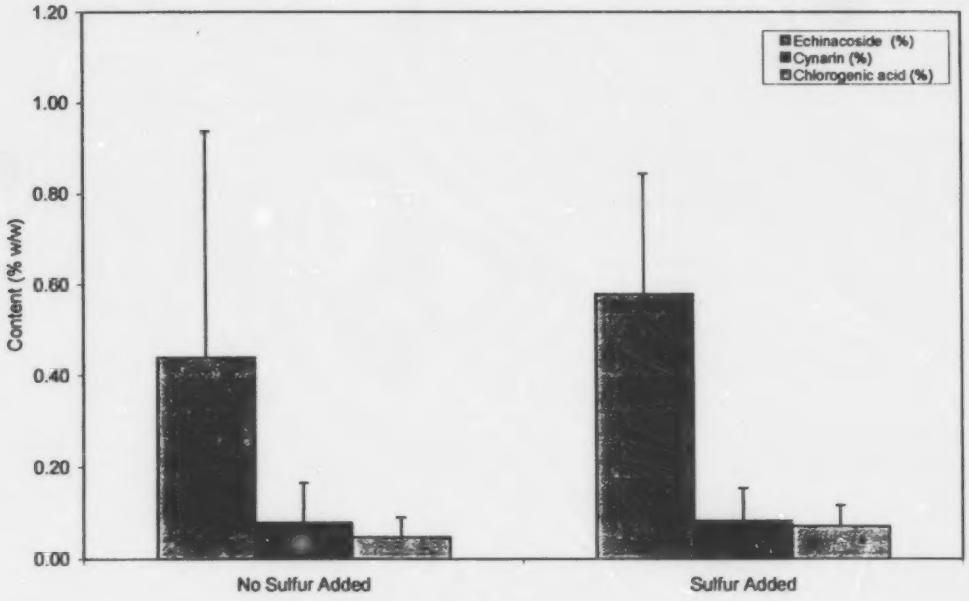


Figure 5. Effect of Sulfur Treatment on Alkylamides Content of One-Year *E. angustifolia* Root Grown at Madras, 1999.

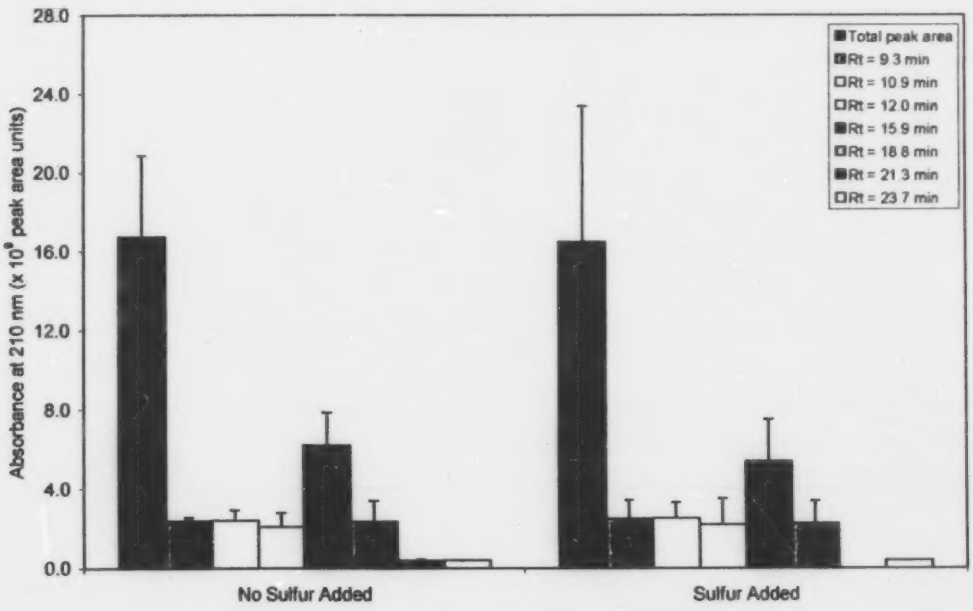


Figure 6. Effect of Aster Yellows Disease on Phenolic Markers Content of One-Year *E. angustifolia* Root, 1999.

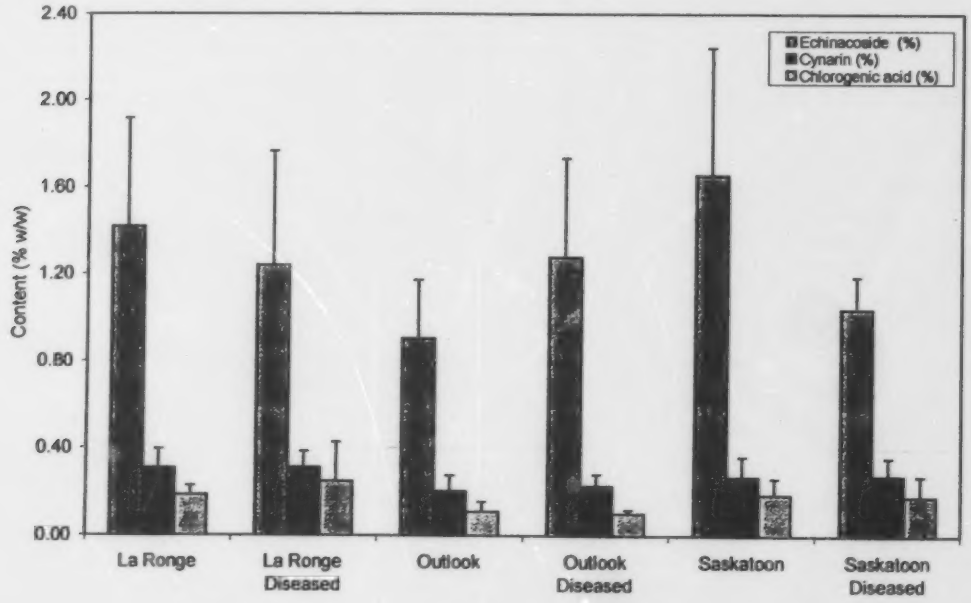
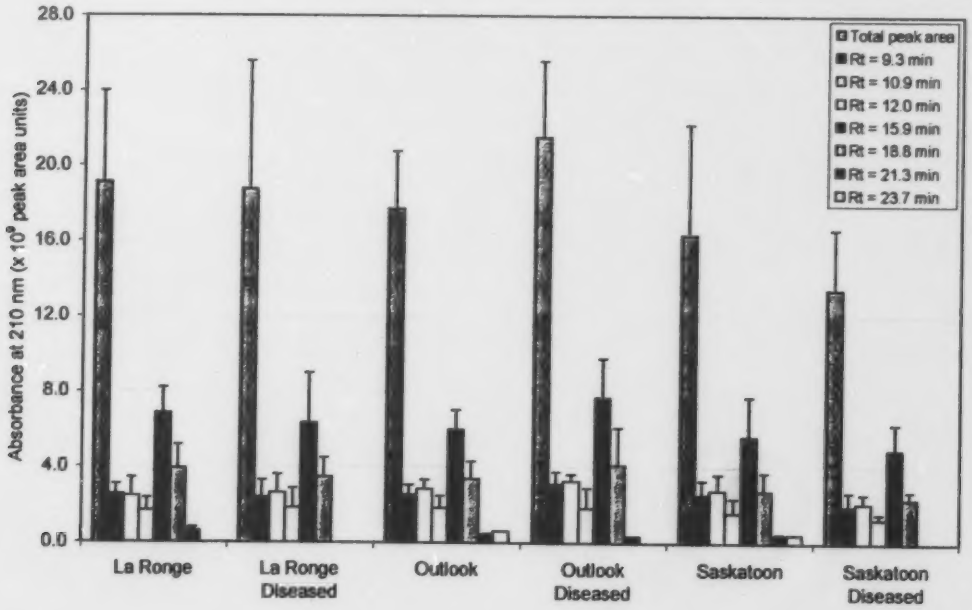


Figure 7. Effect of Aster Yellows Disease on Alkylamides Content of One-Year *E. angustifolia* Root, 1999.



10.0 *Valeriana officinalis* (Valerian)

Valerian is used medicinally for the following therapeutic indications: tenseness, restlessness and irritability, with difficulty in falling asleep. Many chemical constituents have been reported in the literature, but valerenic acids and essential oils are most frequently used as markers for quality determination purposes.

Objectives

1. to determine content of valerenic acid in valerian root by HPLC
2. to determine content of essential oil in valerian root by cohabitual hydrodistillation and to determine content of main volatile compounds in valerian essential oil by GC.

Materials and Methods

Plant Material

In 1999, ninety-eight samples of one-year old dried valerian roots harvested October 15-20 (Saskatchewan), November 15 (Madras) and December 23 (Davis) were provided for testing. Fourteen samples of healthy roots, 2 from each site in Saskatchewan and 4 from each US site were analyzed. Unfortunately, valerian at all 3 Saskatchewan sites suffered heavy losses due to the disease suspected to be aster yellows. Since many diseased plants at various stage of disease development were available, it was decided to examine the effect of aster yellows infection at three stages of symptoms development, described as early (slight yellowing of leaves), well developed (yellowing and purpling of more leaves) and advanced (massive yellowing and purpling of leaves and visible decrease in plant growth), on phytomedicinal quality. A total of 12 samples, 4 from each stage of disease development, were analyzed.

In 2000, one hundred forty four (144) samples of dried valerian root were provided for testing, fifty six (56) were analyzed. Roots from Saskatchewan were one-year old from new planting in 2000, since small number of plants remained healthy at the end of the 1999 season and plots were discontinued. Roots from Madras, Oregon, and Davis, California, were two-year old from 1999 planting. Samples provided by the group conducting agronomy studies in 2000 were the following: 28 samples from each Saskatchewan site consisting of healthy plants and diseased plants at early, medium and advanced stage of disease development; 40 samples from Madras, 10 samples per each of 4 rows; and 20 samples from Davis, California, 5 samples per each of 4 rows. Again this year, valerian at all three Saskatchewan sites suffered losses due to a same disease as observed in 1999 and the effect of disease on root quality was tested for the second consecutive year. Sixteen roots per each of three Saskatchewan sites were tested. Roots from Davis were pooled into two samples from each row, giving 8 samples for analysis. All samples appeared healthy. Samples from Madras, Oregon, suffered long shipment delays at Canadian customs and arrived to Saskatoon moldy. It appeared that roots were not adequately dried prior to shipping and as a results they had to be excluded from testing. All samples for analysis were kept in a dry, dark 10°C storage prior to testing.

Sample Preparation

Each sample of valerian root was cleaned of residual dirt, ground in a hammer mill and passed through a 30 mesh sieve. For the valerenic acid content determination, a sub-sample of

the resulting powder (0.5 g) was extracted with 50 mL of methanol for 30 minutes under reflux. An aliquot of the extract was filtered through a RA 202 filter paper followed by filtering through a 0.45 μm PTFE micro-filter prior to being analyzed for valerenic acid level by high performance liquid chromatography (HPLC). For the essential oil extraction, around 30 grams of ground valerian root, prepared as described above, was extracted by cohabitual hydrodistillation over 4 hours. An aliquot of essential oil was diluted with 2-propanol and subjected to GC analysis.

Liquid Chromatography

The HPLC analysis was performed with a Waters liquid chromatography system comprised of a Waters 600 system controller, Waters 996 diode array detector and Waters 717 Plus autoinjector. Analysis was conducted on a Hewlett-Packard LiChrosorb RP-18 column (200 x 4.6 mm, 5 μm) at 25°C using 20 min isocratic elution and acidified acetonitrile/water as a mobile phase [acetonitrile:water:phosphoric acid (60:40:0.2)]. Flow rate was 1mL/min; injection volume 10 μL. Detection was by UV absorbance at 218 nm. Data was collected and processed digitally with Millennium chromatography software. The calibration curve for valerenic acid was developed using reference standard in the range of 100-800 nanograms per injection. Samples from 2000 harvest were also analyzed for the content of acetoxyvalerenic acid, since pure standard compound became commercially available.

Gas Chromatography (GC)

The GC analysis was performed with a Hewlett Packard HP5890 Series II gas chromatograph and a 0.32 mm x 30 m DB-5 column using flame ionization detection (FID) and helium as a carrier gas at a flow rate of 1.75 mL/min and split ratio of 33:1. After 2 minutes of equilibration at 120°C, the following temperature program was used: 2.5 min @ 120°C, 120°C-200°C (26 min), 5 min @ 200°C, 200°C-280°C (2.6 min), 1 min @ 280°C. The content of bornyl acetate was determined as relative peak area percent. Data was collected and processed digitally with Millennium chromatography software.

Results

A typical HPLC profile for the valerian root extract is presented in Fig. 8. The retention times of acetoxy-valerenic and valerenic acid were consistently at 3.0 and 6.4 minutes, respectively. Minor peaks with retention times at 2.5 min and 8.4 min were suspected to be hydroxy-valerenic acid and valerenal, respectively. Due to the unavailability of these two compounds in pure form, their content could not be included in the analysis. A representative GC profile of valerian root essential oil from Outlook (sample R2-1) is presented in Fig 9.

In 1999, dry roots of one-year valerian root collected at three experimental plots in Saskatchewan contained an average of 0.11% of valerenic acid on dry weight basis (Table 7), which is lower than obtained in a similar study in 1997 (ADF Project #96000172) when average content was 0.20%. Valerian root from Madras contained on average 0.17% and root from Davis 0.06% of valerenic acid. Absolute content of acetoxy-valerenic acid, a derivative of valerenic acid present in appreciable quantities in the root extract (Fig. 8), could not be determined, since no reference standard was commercially available at the time of analysis. However, assuming the same extinction coefficient for both acetoxy-valerenic acid and valerenic acid, the content of acetoxy derivative could be estimated. The sum of all valerenic acid derivatives is often used by the industry as a quality indicator. By following this approach, the

content of valerenic acids was calculated to be 0.26% in Madras, 0.23% in Saskatchewan and 0.14% in Davis. The difference in the length of the growing season (from planting to harvest) between sites should be noted, 177 days in Madras as compared to 138 days in Saskatchewan.

Although causing a significant decrease in the yield of valerian root, aster yellows infection surprisingly has not had an obvious negative impact on valerenic acid content (Table 8). It is possible that the plants stressed by infection continue to synthesize valerenic acid as one of its defense mechanisms.

The valerian root from Saskatchewan had significantly higher essential oil content (0.27%) than valerian root from US sites (0.19% in Madras, 0.15% in Davis), and was on the lower end of the expected range which is 0.3 to 0.7%. The essential oil content was however lower in 1999 than in a similar study conducted in 1997 (ADF Project #96000172), when it was on the high end of the range, 0.78%. In 1997, valerian root from Madras contained only 0.36% essential oil. Considering that phytomedicinal quality of valerian is determined by the content of both valerenic acids and essential oil, these results suggest that Saskatchewan has a competitive advantage in valerian production over Madras, a prime area for herb production in Oregon. Also, composition of oil from all production sites varied considerably. Bornyl acetate content was higher in Saskatchewan (13.3%) than in Madras (11.2%) and Davis (3.6%). Overall, quality of valerian from Saskatchewan and Madras was comparable in 1999, and was significantly superior to the root from Davis.

Results of valerenic acids content in healthy and diseased one-year valerian root grown in 2000 are presented in Table 9 and 10. This year, based on the levels of valerenic acid, quality of valerian root grown at all three Saskatchewan sites was higher (0.17%) than in 1999 (0.11%), but slightly lower than in 1997 (0.19%). Furthermore, valerian from Saskatchewan was if significantly higher quality than valerian from California, which had average valerenic acid content of 0.11%. The same trend was observed in 1999 and 1997. The highest content of valerenic acid in a single root grown in Saskatchewan was 0.42% (La Ronge). Again in 2000, the suspected aster yellows infection did not seem to impact valerenic acids content. No relationship between the stage of disease development and the accumulation of valerenic acid could be made.

It is unfortunate that 2000 valerian root samples from Madras arrived moldy and could not be analyzed. As a result, third year comparison between the quality of Oregon and Saskatchewan grown root could not be conducted. It is also unfortunate that a severe disease problem in Saskatchewan disallowed quality comparison of two-year valerian root. Both one-year and two-year root are used in commerce worldwide. In conclusion, valerian is a promising medicinal root crop for Saskatchewan from quality perspective, but given severe impact of aster yellows disease on root yield and crop survival, it is imperative that advances be made in the management of this disease, before true potential of valerian as a promising medicinal root crop for Saskatchewan can be properly assessed.

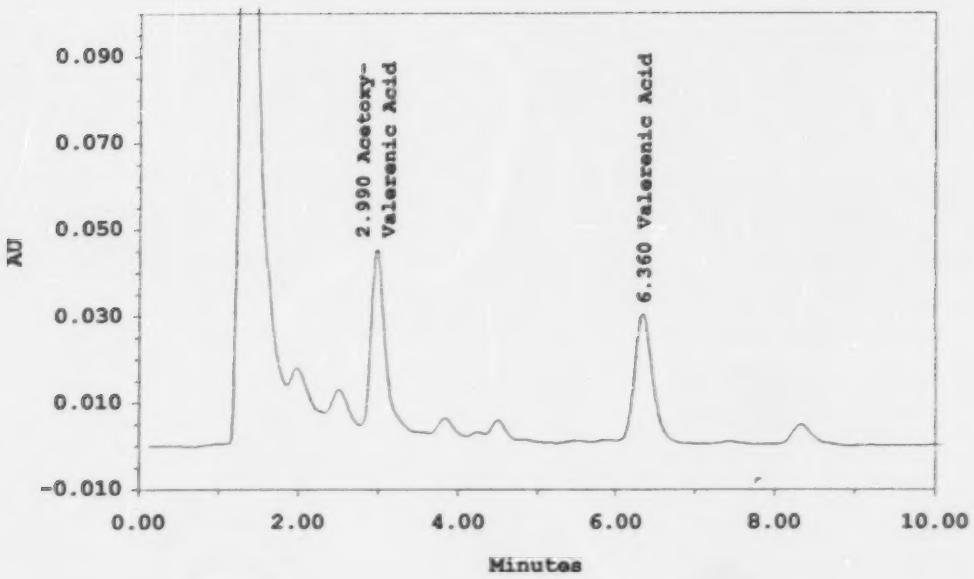


Figure 8. Typical HPLC chromatogram of valerian root extract.

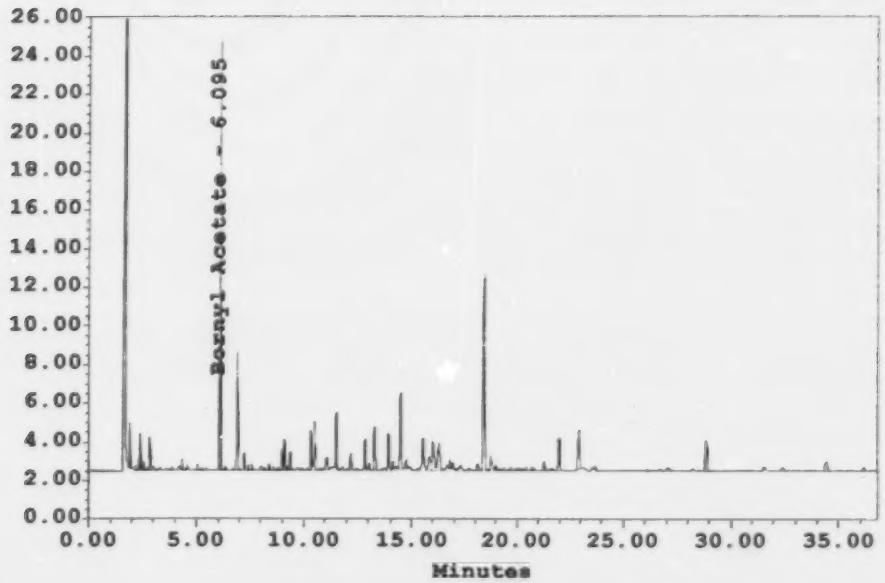


Figure 9. Typical GC chromatogram of valerian root essential oil.

Table 7. Content of Valerenic Acid, Essential Oil and Bornyl Acetate in One-Year Valerian Root Grown in 1999.

Sample ID		Harvest Date	Moisture (%)	Valerenic Acid[1] (%)	Essential Oil[1] (%)	Bornyl Acetate[2] (%)
La Ronge						
	R1	15 Oct	8.15	0.08	0.14	15.7
"	R2	"	8.00	0.01	0.17	4.1
Outlook						
"	R1	18 Oct	8.42	0.13	0.30	16.8
"	R2	"	8.51	0.13	0.40	17.6
Saskatoon						
"	R1	20 Oct	8.33	0.13	0.22	8.4
"	R2	"	8.49	0.15	0.37	17.1
All 3 sites Mean±S.D.				**0.11±0.05**	**0.27±0.11**	**13.3±5.7**
Madras						
"	R1	15 Nov	6.85	0.13	0.14	3.7
"	R2	"	6.15	0.18	0.30	18.7
"	R3	"	6.25	0.26	0.18	15.9
"	R4	"	6.76	0.13	0.13	6.5
Mean±S.D.				**0.17±0.06**	**0.19±0.08**	**11.2±7.2**
Davis						
"	R1	23 Dec	6.39	0.07	0.07	4.7
"	R2	"	6.59	0.08	0.17	4.2
"	R3	"	7.13	0.08	0.18	1.7
"	R4	"	6.57	nd[3]	0.16	4.0
Mean±S.D.				**0.06±0.03**	**0.15±0.05**	**3.6±1.3**

[1]Contents of valerenic acid and essential oil in the root are reported on dry weight basis

[2]Bornyl acetate is reported on a relative basis, as peak area percent of total essential oils; [3]nd - not detected

Table 8. Effect of Aster Yellows Infection on Valerenic Acid Content of One-Year Valerian Root Grown in , 1999.

Sample ID	Stage of Disease Development		Harvest Date	Moisture (%)	Valerenic Acid[1] (%)
Outlook	R1-1	Early	18 Oct	9.89	0.18
	R2-1	"	"	9.50	0.20
	R1-1	Well Developed	"	10.62	0.14
	R2-1	"	"	10.15	0.09
	R1-1	Advanced	"	11.93	0.26
	R2-1	"	"	11.93	0.08
Saskatoon	R1-1	Early	20 Oct	9.86	0.13
	R2-1	"	"	9.66	0.34
	R1-1	Well Developed	"	10.23	0.10
	R2-1	"	"	9.78	0.09
	R1-1	Advanced	"	9.97	0.61
	R2-1	"	"	9.97	0.02

[1]Content of valerenic acid in the root is reported on dry weight basis

Table 9. Effect of Aster Yellows Infection on Valerenic Acid Content of One-Year Valerian Root Grown in 2000.

Sample ID		Stage of Disease Development	Moisture (%)	Acetoxy-valerenic Acid (% w/w)	Valerenic Acid (% w/w)	Total Valerenic Acid (% w/w)
Saskatoon	R1	Healthy	11.04	0.20	0.09	0.29
"	R2	"	10.74	0.13	0.10	0.23
"	R3	"	11.29	0.02	0.21	0.23
"	R4	"	11.13	0.19	0.22	0.41
Mean±S.D.				**0.14±0.09**	**0.16±0.07**	**0.29±0.08**
"	R1	Early	11.11	0.24	0.17	0.40
"	R2	"	11.24	0.19	0.14	0.33
"	R3	"	11.43	0.12	0.15	0.28
"	R4	"	11.73	0.17	0.13	0.30
Mean±S.D.				**0.18±0.05**	**0.15±0.02**	**0.33±0.05**
"	R1	Well developed	11.14	0.09	0.15	0.25
"	R2	"	10.58	0.13	0.12	0.25
"	R3	"	10.88	0.20	0.11	0.31
"	R4	"	11.52	0.06	0.19	0.25
Mean±S.D.				**0.12±0.06**	**0.14±0.04**	**0.27±0.03**
"	R1	Advanced	11.06	0.20	0.12	0.32
"	R2	"	11.02	0.19	0.17	0.36
"	R3	"	11.11	0.20	0.14	0.34
"	R4	"	10.10	0.21	0.11	0.32
Mean±S.D.				**0.20±0.01**	**0.13±0.03**	**0.34±0.02**
Outlook	R1	Healthy	11.26	0.27	0.08	0.35
"	R2	"	12.01	0.02	0.27	0.29
"	R3	"	10.94	0.09	0.15	0.24
"	R4	"	11.59	0.09	0.26	0.35
Mean±S.D.				**0.12±0.11**	**0.19±0.09**	**0.31±0.05**
"	R1	Early	10.64	0.12	0.13	0.25
"	R2	"	11.00	0.08	0.08	0.16
"	R3	"	11.00	0.14	0.13	0.27
"	R4	"	11.00	0.18	0.14	0.32
Mean±S.D.				**0.13±0.04**	**0.12±0.03**	**0.25±0.07**
"	R1	Well developed	10.98	0.17	0.08	0.25
"	R2	"	10.89	0.07	0.09	0.16
"	R3	"	12.16	0.08	0.21	0.29
"	R4	"	10.88	0.26	0.23	0.49
Mean±S.D.				**0.15±0.09**	**0.15±0.08**	**0.30±0.14**
"	R1	Advanced	11.38	0.29	0.07	0.36
"	R2	"	10.81	0.20	0.06	0.26
"	R3	"	12.56	0.14	0.05	0.19
"	R4	"	11.40	0.07	0.18	0.25
Mean±S.D				**0.17±0.09**	**0.09±0.06**	**0.26±0.07**

Table 10. Effect of Aster Yellows Infection on Valerenic Acid Content of One-Year Valerian Root Grown in 2000 (continuation of Table 9).

Sample ID	Stage of Disease Development	Moisture (%)	Acetoxy-valerenic Acid (% w/w)	Valerenic Acid (% w/w)	Total Valerenic Acid (% w/w)
La Ronge R1	Healthy	11.00	0.16	0.26	0.42
" R2	"	11.48	0.12	0.15	0.27
" R3	"	10.69	0.24	0.09	0.33
" R4	"	11.24	0.10	0.08	0.18
Mean±S.D			**0.16±0.06**	**0.15±0.09**	**0.31±0.10**
" R1	Early	11.39	0.13	0.11	0.24
" R2	"	10.50	0.07	0.14	0.21
" R3	"	11.20	0.10	0.23	0.33
" R4	"	9.83	0.21	0.10	0.31
Mean±S.D			**0.13±0.06**	**0.14±0.06**	**0.27±0.06**
" R1	Well developed	11.30	0.20	0.09	0.29
" R2	"	11.29	0.12	0.23	0.35
" R3	"	10.77	0.16	0.11	0.27
" R4	"	10.30	0.15	0.10	0.25
Mean±S.D			**0.16±0.03**	**0.13±0.06**	**0.29±0.04**
" R1	Advanced	11.39	0.19	0.11	0.30
" R2	"	10.60	0.14	0.17	0.31
" R3	"	12.82	0.25	0.10	0.35
" R4	"	12.42	0.19	0.16	0.35
Mean±S.D			**0.19±0.05**	**0.13±0.04**	**0.33±0.03**
UC Davis R1	Healthy	9.20	0.14	0.14	0.28
" R2	"	9.53	0.14	0.11	0.26
" R3	"	9.17	0.06	0.14	0.21
" R4	"	9.41	0.11	0.05	0.16
" R5	"	8.84	0.23	0.08	0.31
" R6	"	9.74	0.10	0.06	0.16
" R7	"	9.04	0.06	0.21	0.27
" R8	"	8.89	0.20	0.10	0.30
Mean±S.D			**0.13±0.06**	**0.11±0.05**	**0.24±0.06**

11.0 *Arctium lappa* (Burdock)

Burdock has been used in folk medicine as a diuretic, as a blood purifier, as a laxative, an apetite stimulant, in rheumatic complaints, as well as externally for skin disorders. Root extracts have been reported in traditional systems of medicine to have antimicrobial activity. Many chemical constituents of burdock, such as 1.9-3.65% of polyphenols including chlorogenic acid, have been reported in the literature. Other major constituents are carbohydrates, primarily inulin.

Objective

1. to determine chlorogenic acid content in burdock root by HPLC.

Materials and Methods

Plant Material

In 1999, burdock production failed at all Saskatchewan and US sites due to low germination rate of burdock seed. Consequently, no burdock samples were provided for analysis. In 2000, seventy nine samples of dry one-year burdock root, 4 samples per each of 4 rows per site, from each of 5 sites were provided to the Herb Research Program for testing. Samples from rows 1 and 2, and rows 3 and 4 were combined for each site bringing the total number of samples for analysis to 40, eight per each site.

Sample Preparation

Each sample of entire burdock root was cleaned of residual dirt, ground in a hammer mill and passed through a 30 mesh sieve. A sub-sample of the resulting powder was extracted with methanol for 30 minutes under reflux. An aliquot of the extract was filtered through a 0.45 μm microfilter and subjected to HPLC analysis.

Liquid Chromatography

An HPLC analysis was performed with the same instrument and under the same conditions as described for phenolic compounds in echinacea. Chlorogenic acid was used as a standard. Data was collected and processed digitally with Millennium chromatography software.

Results

The HPLC profile and UV spectra of phenolic acids in burdock root extract are presented on Figure 10. The UV spectra of all four main compounds with retention times RT= 9.3; 19.6; 20.8; and 21.6 min indicate that they have the same absorption maximum, 328 nm, which suggests that compounds belong to the same class of natural products. In addition to chlorogenic acid, isochlorogenic acid, caffeic acid, and other caffeic acid derivatives have been reported in burdock root in the literature.

The content of chlorogenic acid in one-year burdock root grown at three Saskatchewan locations and two US locations in 2000 is presented in Table 11 and 12, respectively. This is the first year of testing burdock for Northern Vigor® response. The chlorogenic acid content varied from an average of 0.32% in Davis to 0.82% in La Ronge. These results indicate that Saskatchewan grown root is of higher quality. Root grown in Saskatchewan contained on average 0.73% chlorogenic acid, which is 228% the content in Madras and Davis grown root. The content of three other compounds detected in burdock root (Figure 10a), likely various phenolic acids related to chlorogenic acid, was also proportionally higher in roots from Saskatchewan. Overall, these data suggest a strong possibility of a Northern Vigor® response with respect to phytomedicinal quality of burdock root.

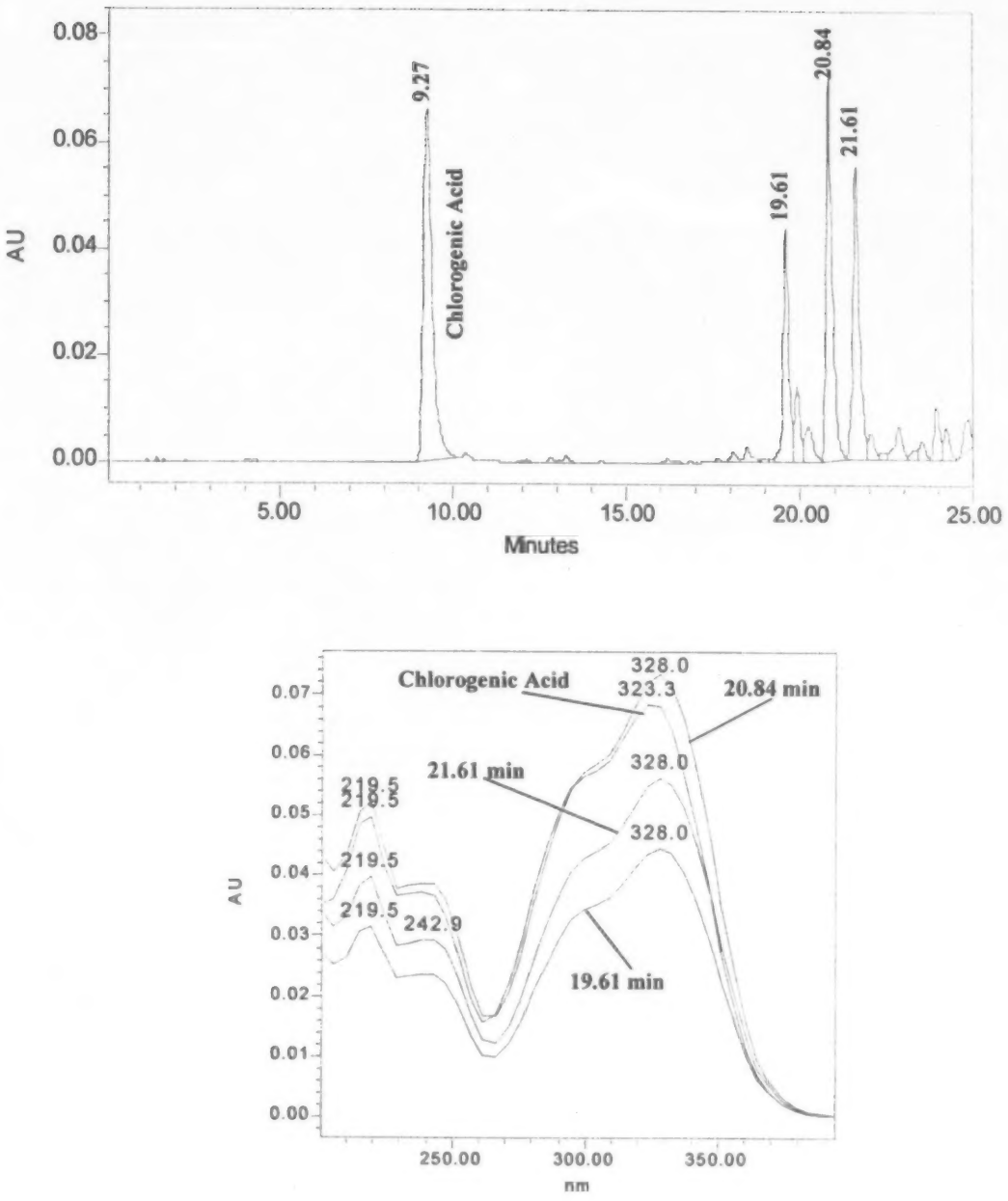


Figure 10. Typical HPLC profile (a) and UV spectra (b) of burdock root extract.

Table 11. Content of Chlorogenic Acid in One-Year Burdock Root Grown at Saskatchewan Sites in 2000.

Sample ID	Harvest Date	Moisture (%)	Chlorogenic acid[1] (%)	Chlorogenic acid Mean±S.D. (%)
La Ronge R1-1	24 October	11.44	1.03	
R1-2	"	9.90	0.71	
R2-1	"	10.89	0.65	
R2-2	"	10.61	0.66	
R3-1	"	10.91	0.79	
R3-2	"	10.09	0.83	
R4-1	"	9.45	1.18	
R4-2	"	9.78	0.69	
				0.82±0.19
Outlook R1-1	20 October	11.25	0.68	
R1-2	"	9.32	0.73	
R2-1	"	9.68	0.55	
R2-2	"	9.73	0.75	
R3-1	"	9.08	0.64	
R3-2	"	9.32	0.81	
R4-1	"	9.77	0.65	
R4-2	"	10.00	0.62	
				0.68±0.08
Saskatoon R1-1	19 October	10.01	0.75	
R1-2	"	11.11	0.47	
R2-1	"	9.16	0.81	
R2-2	"	10.04	0.65	
R3-1	"	9.77	0.95	
R3-2	"	9.75	0.88	
R4-1	"	10.50	0.56	
R4-2	"	11.60	0.48	
				0.69±0.1

[1]Content of chlorogenic acid is reported on dry weight basis

Table 12. Content of Chlorogenic Acid in One-Year Burdock Root Grown at US Sites in 2000.

Sample ID		Harvest Date	Moisture (%)	Chlorogenic acid[1] (%)	Chlorogenic acid Mean±S.D. (%)
Madras, OR	R1-1	3 November	12.09	0.18	
"	R1-2	"	12.27	0.54	
"	R2-1	"	8.38	0.17	
"	R2-2	"	7.33	0.42	
"	R3-1	"	8.16	0.27	
"	R3-2	"	8.20	0.52	
"	R4-1	"	8.71	0.30	
"	R4-2	"	8.04	0.26	
					0.33 ±0.14
Davis, CA	R1-1	23 October	8.91	0.30	
"	R1-2	"	10.73	0.24	
"	R2-1	"	10.73	0.27	
"	R2-2	"	10.93	0.24	
"	R3-1	"	10.91	0.24	
"	R3-2	"	10.68	0.28	
"	R4-1	"	12.51	0.42	
"	R4-2	"	10.42	0.56	
					0.32±0.11

[1]Content of chlorogenic acid is reported on dry weight basis

12.0 *Nepeta cataria* (Catnip)

Catnip is a source of essential oil used for its calming and sedative effect in aromatherapy. It is also used in fragrance formulations and has a variety of other industrial applications.

Objectives

1. to determine content and composition of catnip essential oil by GC
2. to determine a distribution of essential oil in different plant parts of catnip

Material and Methods

Plant Material

In 1999, thirty-two samples of dried catnip plants (entire above ground parts) harvested August 18-20 in Saskatchewan, July 27 in Madras, Oregon, and August 18 in Davis, California, were provided to the Herb Research Program for testing. A total of 20 samples, 4 from each site, were analyzed. Three catnip plants from Saskatoon and Outlook were separated into leaves, flowers and stems in order to examine a composition of essential oil in various plant parts. Flowers, leaves and stems were pooled from each location to give total of 6 samples, and were subjected to GC analysis.

In 2000, dried top half of each catnip plant, 10 plants per row, total of 40 plants, were provided from each of 3 Saskatchewas sites. Twelve samples, 3 per row, were provided from each of 2 US sites bringing to total number of samples to 144. One plant per row, 4 per site, from each of five harvest sites were used for testing. A total of twenty samples, four from each site, were analyzed. In addition, one catnip plant from each of Saskatoon, Outlook and La Ronge was separated into flowers and leaves together (it was impossible to separate leaves from flowers of dry samples), and stems, in order to examine the distribution and composition of essential oil in various plant parts. Also, four large composite samples of catnip were sent to Crop Diversification South in Brooks, Alberta, for distillation in a pilot scale (pseudo-commercial scale) distillation unit. In order to enable results comparison of laboratory and pilot scale distillation, small sub-sample of each of these 4 composite samples was ground and kept for laboratory scale distillation and GC analysis by the Herb Research Program.

Sample Preparation

For analysis of essential oil content and composition, each plant sample was ground in a hammer mill, and a sub-sample of approximately 100 g of ground powder was extracted by hydrodistillation over 4 hrs. Given that density of catnip essential oil and water are similar, majority of oil was collected on the top of a water layer in a hydrodistillation apparatus receiver, but some escaped to the bottom of the receiver and had to be recovered by diethyl ether. Essential oil collected either way was stored separately at -18°C until further analysis by GC.

Gas Chromatography (GC)

The GC work was performed on a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a HP-5 (30 m x 0.32 mm, 0.25 μm film thickness) fused-silica capillary column (crosslinked 5% diphenyl - 95% dimethylsiloxane copolymer) (Waters). The carrier gas was helium operating at 1.67 mL/min and a split ratio of

33:1. The inlet and detector temperatures were set at 250°C. The following temperature program was used: initial temperature 70°C for 2.50 min, elevated to 200°C at a rate of 5°C/min, kept at 250°C for 5 min, elevated to 280°C at 30°C/min, kept at 280°C for 1 min.

Each essential oil sample was dissolved in hexane (10 μL in 990 μL) and 1 μL was injected into the column. Data were collected and processed with Millennium chromatography software. Compounds of interest, nepetalactone, caryophyllene and caryophyllene oxide, were positively identified by gas chromatography-mass spectroscopy (GC-MS) in our previous studies. The relative content of each compound was expressed as a peak area over the total peak area (relative area %).

Results

In 1999, the amount of essential oil and the content of three main oil constituents obtained from catnip grown in 1999 at all 5 test sites is presented in Table 13. This year the entire catnip plan was cut for testing. On average, oil content was lower (0.09% to 0.2%) than obtained in a similar study conducted in 1997, when it ranged from 0.29% to 0.37% for Saskatchewan sites. A likely explanation for this finding is the fact that the entire catnip plants were harvested and analyzed in1999, while only flowering tops (flower heads and leaves) of catnip plants were used for analysis in 1997. Heavy stems contribute greatly to the weight of catnip plants cut at ground level, but are most frequently essential oil free. Therefore, an inclusion of stems into plant sample for analysis, had greatly impacted low essential oil levels in 1999. It should be mentioned that to the best of our knowledge catnip grown on large scale for commercial production of essential oil is typically cut close to the ground level.

Further insight into the distribution of catnip essential oil between flowers, leaves and stems is provided in Table 14. Flowers from both Saskatoon and Outlook contained the highest amount of essential oil, 0.69%-0.84%, and oil's main constituent, nepetalactone (93.8%-95.6%). Leaves contained 0.16%-0.18% essential oil and 72-76% nepetalactone, while stems contained none. Since stems have no value for essential oil extraction from catnip, large scale harvesting of catnip will greatly depend on the height of the cut. Catnip shows a good potential as an aromatic plant for Saskatchewan, but results of this study suggest that close attention need to be paid to catnip commercial harvesting practice, particularly height of the cut.

The yield of catnip essential oil and a content of its major volatile compounds, obtained from 2000 harvest year is presented in Table 15. Oil content varied from 0.04 % in Davis to 0.2% in Madras. Among Saskatchewan samples, catnip from Saskatoon yielded 0.14% essential oil, followed by Outlook at 0.13% and La Ronge at 0.07%. It should be noted that in Madras, catnip was harvested a month earlier than in La Ronge, which might have contributed to the loss of essential oil in catnip from La Ronge. It is well documented that harvest time is a very important consideration for aromatic plants, given the impact it has on both yield and composition of essential oil. Nepetalactone, a major volatile compound in catnip oil accounted for 55.3% to 78.5% of the entire oil, which is slightly lower percentage than last year, but is within the expected range. Distribution of catnip essential oil between flowers and leaves, and stems is presented in Table 16. Combined flower and leaves sample yielded on average 0.28% of essential oil. Flowers and leaves were confirmed to be main sources of essential oil. Last year, flowers contained 0.76% and leaves 0.17% of essential oil. Since stems contribute greatly to the

Table 13. Content of Major Volatile Compounds in Essential Oil of Catnip Grown in 1999.

Sample ID		Harvest Date	Essential Oil[1] (%)	Nepetalactone[2] (%)	Caryophyllene[2] (%)	Caryophyllene oxide[2] (%)
La Ronge	R1	18 Aug	0.16	82.16	4.02	3.11
	R2	"	0.06	68.87	6.19	8.89
	R3	"	0.06	62.86	1.89	9.90
	R4	"	0.08	81.54	4.35	6.53
	Mean±S.D.		**0.09±0.05**	**73.86±9.55**	**4.11±1.76**	**7.11±3.02**
Outlook	R1	20 Aug	0.08	76.14	1.28	10.13
	R2	"	0.11	85.34	3.21	3.65
	R3	"	0.04	53.15	2.91	16.18
	R4	"	0.17	89.82	3.39	2.65
	Mean±S.D.		**0.10±0.05**	**76.11±16.33**	**2.70±0.97**	**8.15±6.30**
Saskatoon	R1	20 Aug	0.20	78.23	2.07	5.38
	R2	"	0.21	90.42	1.75	2.51
	R3	"	0.12	74.62	2.13	6.98
	R4	"	0.29	89.90	2.26	1.97
	Mean±S.D.		**0.20±0.07**	**83.29±8.07**	**2.05±0.22**	**4.21±2.38**
Madras	R1	27 Jul	0.12	67.12	4.19	9.63
	R2	"	0.04	19.10	1.94	23.32
	R3	"	0.22	80.88	3.78	3.72
	R4	"	0.29	86.92	3.77	2.85
	Mean±S.D.		**0.16±0.11**	**63.51±30.74**	**3.42±1.01**	**9.88±9.45**
Davis	R1-1	18 Aug	0.26	89.29	4.87	2.06
	R2-1	"	0.12	51.19	7.02	8.19
	R3-1	"	0.29	89.16	3.92	2.37
	R4-1	"	0.13	51.32	7.95	9.94
	Mean±S.D.		**0.20±0.09**	**70.24±21.92**	**5.94±1.86**	**5.64±4.02**

[1] Content of essential oil is reported on as is basis

[2] Content of essential oil components are reported on a relative basis, as peak area percent

Table 14. Content of Major Essential Oil Compounds in Catnip Plant Parts Grown in 1999.

Sample ID		Sample Type	Harvest Date	Essential Oil[1] (%)	Nepetalactone[2] (%)	Caryophyllene[2] (%)	Caryophyllene oxide[2] (%)
Outlook	R1	Stems	20 Aug	0.003	‡	‡	‡
Saskatoon	R1	"	"	0.006	‡	‡	‡
Outlook	R1	Leaves	"	0.16	72.00	4.25	6.72
Saskatoon	R1	"	"	0.18	76.02	5.74	7.13
Outlook	R1	Flowers	"	0.69	95.57	2.04	1.37
Saskatoon	R1	"	"	0.84	93.84	2.89	1.31

[1]Content of essential oil is reported on as is basis; ‡ insufficient sample for analysis

[2]Content of essential oil components are reported on a relative basis, as a peak area percent

Table 15. Content of Major Volatile Compounds in Essential Oil of Catnip in 2000.

Sample ID		Essential Oil[1] (%)	Nepetalactone[2] (%)	Caryophyllene[2] (%)	Caryophyllene oxide[2] (%)
Saskatoon	R1	0.21	82.04	8.33	3.78
"	R2	0.14	66.06	4.29	8.64
"	R3	0.07	42.03	11.97	19.34
"	R4	0.14	64.48	5.75	9.20
Mean ±S.D.		**0.14±0.06**	**63.65±16.45**	**7.59±3.37**	**10.24±6.54**
Outlook	R1	0.08	65.88	10.62	7.30
"	R2	0.11	64.67	15.04	6.76
"	R3	0.21	86.85	4.92	3.00
"	R4	0.14	80.56	8.74	4.28
Mean ± S.D.		**0.13±0.06**	**74.49±10.96**	**9.83±4.21**	**5.34±2.04**
La Ronge	R1	0.07	79.53	7.58	6.92
"	R2	0.07	-	-	-
"	R3	0.06	nd[3]	56.41	43.59
"	R4	0.07	31.14	22.62	17.89
Mean ± S.D.		**0.07±0.01**	**55.34±34.22**	**28.87±25.00**	**22.80±18.82**
Davis, CA,	R1	0.04	49.15	9.20	14.95
"	R2	0.04	53.93	8.71	13.71
"	R3	0.04	75.72	2.64	8.25
"	R4	0.02	71.65	7.00	9.97
Mean ± S.D.		**0.04±0.01**	**62.61±13.04**	**6.89±2.98**	**11.72±3.14**
Madras, OR,	R1	0.23	71.24	3.95	6.74
"	R2	0.08	75.30	12.69	7.08
"	R3	0.13	71.84	15.56	6.86
"	R4	0.36	95.6	nd	4.40
Mean±S.D.		**0.20±0.12**	**78.50±11.54**	**10.73±6.05**	**6.27±1.25**

[1]Content of essential oil is the sum of oil collected and oil extracted from distillate with diethyl ether

[2]Contents of essential oil components are reported on a relative basis, as peak area percent

[3]nd-not detected

Table 16. Content of Major Essential Oil Compounds in Catnip Plant Parts Grown in 2000.

Sample ID	Sample Type	Essential Oil (%)	Nepetalactone[1] (%)	Caryophyllene[1] (%)	Caryophyllene oxide[1] (%)
	Stems				
Saskatoon	"	0.003[2]	nd[3]	9.83	40.68
Outlook	"	0.004[2]	33.37	nd	14.59
La Ronge	"	0.003[2]	nd	12.94	38.73
	Flowers and Leaves				
Saskatoon	"	0.29[4]	76.88	8.96	5.45
Outlook	"	0.27	61.09	9.98	5.81
La Ronge	"	0.28	75.89	10.91	6.06

[1]Content of volatile compounds in essential oil is reported on a relative basis, as a peak area percent
[2]Content of essential oil extracted from distillate with diethyl ether
[3]nd-not detected
[4]Content of essential oil is the sum of oil collected and oil extracted from distillate with diethyl ether

weight of catnip samples and are for all practical purposes essential oil free, at least thick main stems should be removed prior to distillation if feasible.

The GC profile of catnip essential oil showing major constituents, nepetalactone, caryophyllene and caryophyllene oxide, is presented in Fig. 11 and 12. The GC profile of oil obtained on a pilot scale steam distillation unit at CDC-South in Brooks, Alberta (Fig. 11), shows a very slight difference in oil composition in comparison to oil obtained on a laboratory scale. This difference is not of a commercial significance. However, the yields of oil extracted on a pilot scale unit were lower than the yields obtained on a laboratory unit. This is to be expected and should be taken into account if one contemplates large scale distillation of oil from catnip.

The two year study aimed at probing latitudinal and environmental effect on quantity and quality of essential oil from catnip provided no encouraging results. It does not appear that Saskatchewan has any competitive advantage over Oregon or California in growing catnip. Our data indicated however that seasonal influences, harvest time and practice, particularly with regard to which plant part to harvest and post-harvest handling are of great importance for commercial production of catnip for essential oil.

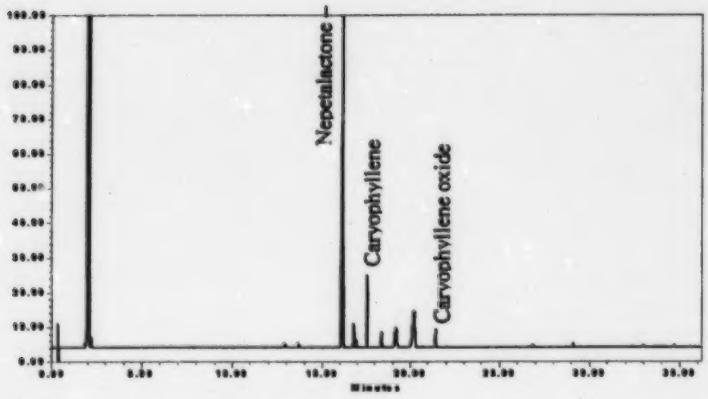


Figure 11. Typical GC chromatogram of catnip essential oil obtained on a pilot scale steam distillation unit at CDC-South in Brooks, Alberta.

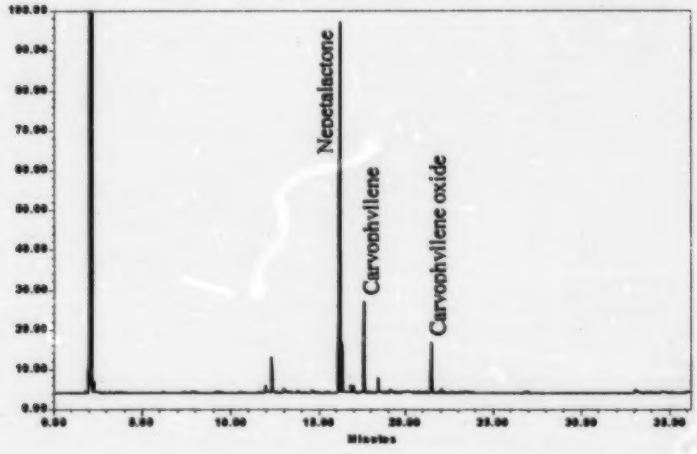


Figure 12. Typical GC chromatogram of catnip essential oil obtained in a laboratory distillation unit.

13.0 *Calendula officinalis* (Calendula)

Calendula is used medicinally for the following therapeutic indications: inflammations of the skin and mucosa, and as an aid to wound healing. Many chemical constituents have been reported in the literature, but flavonoids are most frequently used as markers for quality determination purposes.

Objective

1. to establish flavonoid profile and determine content of major flavonoid compounds, specifically flavonol glycoside markers such as isorhamnetin-3-glucoside (I-3-G), isorhamnetin-3-rutinoside (I-3-R) and isorhamnetin-3-O-2-rhamnosyl-rutinoside (I-3-R-R) in extracts of calendula flowers by HPLC.

Materials and Methods

Plant Material

In 1999, flowers were harvested once per week or biweekly from late June/early July until late September, resulting in collection of 272 samples from 5 sites. A total of 60 samples of dried calendula flowers (12 samples from each of 5 sites, collected at 3 harvest dates, once in July, August and September), were analyzed. Selection on samples was based on sample size and healthy appearance. Samples from La Ronge collected on 13 July, 27 July, 3 August, 10 August, 23 August and 7 September were partially moldy, likely due to difficulties in facilitating sample drying and transport from La Ronge to Saskatoon. These samples were eliminated from the sample pool for testing and had a bearing on the sample selection for analysis from remaining four sites.

In 2000, flowers were harvested once per week or biweekly from middle to late July until middle of September, resulting in collection of 200 samples from 5 sites. Samples from rows 1 and 2, and rows 3 and 4 were combined for each site and each harvest time. Total of 30 samples of dried calendula flowers (6 samples from each of 5 sites, collected at 3 harvest dates, once each in July, August and September, with the exception of La Ronge which first harvested on August 8) were analyzed. Selection on samples was based on sample size and healthy appearance.

Sample Preparation

Dried flower heads were ground in a coffee grinder, and a sub-sample of the resulting powder (0.5 g) was extracted with 50% (v/v) methanol on a water bath shaker at 30°C over 24 hours. The extracts were filtered through a Whatman No. 1 filter paper to remove particulate matter. A sub-sample of the resulting extract was filtered through a 0.45 µm microfilter prior to being analyzed for flavonol glycoside content using HPLC.

Liquid Chromatography

An HPLC analysis of flavonol glycosides was performed with a Waters system comprised of a Waters 600 system controller, Waters 996 diode array detector and Waters 717 Plus autoinjector. Analysis was conducted by isocratic elution on a Waters Novapak C-18 column (150 x 3.9 mm, 5 µm) at 25°C using mobile phase consisting of 14% 2-propanol, 5%

tetrahydrofuran and 81% water; flow rate was 1.2 mL/min and UV detection at 356 nm. Data was collected and processed digitally with Millennium chromatography software. Isorhamnetin-3-rutinoside (I-3R) and isorhamnetin-3-glucoside (I-3G) were calibrated using the reference standard in the range of 50-1000 nanograms per injection, and 5-100 nanograms per injection, respectively. The I-3-RR (isorhamnetin-3-rhamnosylrutinoside) was positively identified in our earlier studies of calendula extract by LC-MS. In the absence of I-3-RR standard, the content of I-3-RR was calculated using the standard curve for I-3R after correction for the molecular weights.

Results

Ground samples and intact dry flowers of calendula harvested in 2000 were evaluated for differences in color and appearance. Harvest time had an obvious effect on color of ground samples. The color from 1st harvest time was bright orange and comparable for all five sites. Flowers from later harvests were visibly darker (notably for Davis), with exception of Madras sample, where change was slight. Evaluation of intact flowers revealed that flowers from 1st harvest were the largest across all five sites. It appeared that Oregon flowers had more petals than flowers from other locations. Flowers from California, especially from 2nd and 3rd harvest, were the smallest of all the samples. It is possible that plants in Davis were affected by aster yellows that resulted in smaller size flowers with dull yellow petals, and/or flowers were collected beyond full bloom stage.

The content of three flavonol glycosides, isorhamnetin-3-O-2-rhamnosyl-rutinoside (I-3-RR), isorhamnetin-3-rutinoside (I-3R) and isorhamnetin-3-glucoside (I-3G) in calendula flowers collected at 5 study sites at 3 harvest dates is presented in Table 17 (Saskatchewan sites) and Table 18 (US sites). The total flavonol glycosides content expressed as sum of I-3RR, I-R and I-3G, ranged from 0.8% in Davis to 1.43% in Madras. Flowers from all three Saskatchewan sites were of comparable quality with levels of 1.03%. Two main compounds were I-3-RR and I-3R. In Madras, harvest was at least two weeks earlier (July 6) than at other sites, while in La Ronge no July harvest was conducted. Given that flavonol glycosides content is the highest in petals in early blooming stage (pigment has a specific function in attracting the pollinating insects), an early harvest in Madras may partially explain the high biomarkers level detected there. As in 1999 and our earlier studies, the level of flavonol glycosides decreased progressively from July to September; it was up to twice as high in the middle of July as it was in the middle of September.

The HPLC profile and the UV spectra of flavonol glycosides in calendula flowers collected in full bloom are presented in Figure 13. Peaks with retention times 2.25 min, 4.67 min and 5.95 min are I-3-RR, I-3R and I-3G, respectively. The remaining peaks could not be assigned at present, but are likely other isorhamnetin derivatives, or quercetin derivatives.

Table 17. Content of Dominant Flavonoid Compounds in Calendula Flowers Grown in Saskatchewan in 2000.

Sample ID		Harvest Date	Moisture (%)	I-3-R-R[1,2] (%)	I-3-R[1] (%)	I-3-G[1] (%)	Total Flavonoids[3] (%)
La Ronge	R1, R2	8 August	8.93	0.51	0.41	0.03	0.95
"	R3, R4	"	9.21	0.50	0.48	0.03	1.01
"	R1, R2	24 August	8.86	0.45	0.46	0.03	0.94
"	R3, R4	"	9.46	0.43	0.51	0.04	0.98
"	R1, R2	13 September	10.28	0.50	0.69	0.04	1.23
"	R3, R4	"	9.83	0.39	0.64	0.06	1.09
Mean±S.D.				**0.46±0.05**	**0.53±0.11**	**0.04±0.01**	**1.03±0.11**
Outlook	R1, R2	24 July	9.58	0.74	0.48	0.04	1.26
"	R3, R4	"	9.51	0.72	0.50	0.04	1.26
"	R1, R2	14 August	9.33	0.44	0.35	0.02	0.81
"	R3, R4	"	10.09	0.52	0.43	0.03	0.98
"	R1, R2	13 September	9.53	0.38	0.53	0.04	0.95
"	R3, R4	"	9.65	0.38	0.54	0.04	0.96
Mean±S.D.				**0.53±0.16**	**0.47±0.07**	**0.03±0.01**	**1.03±0.01**
Saskatoon	R1, R2	25 July	9.13	0.80	0.50	0.04	1.34
"	R3, R4	"	9.35	0.67	0.50	0.04	1.21
"	R1, R2	15 August	9.85	0.54	0.44	0.04	1.02
"	R3, R4	"	10.70	0.51	0.36	0.03	0.90
"	R1, R2	12 September	10.09	0.35	0.43	0.04	0.82
"	R3, R4	"	9.31	0.37	0.48	0.04	0.89
Mean±S.D.				**0.54±0.17**	**0.45±0.05**	**0.04±0.00**	**1.03±0.20**

[1]Content of I-3-R-R (isorhamnetin-3-O-2-rhamnosyl-rutinoside), I-3-R (isorhamnetin-3-rutinoside) and I-3-G (isorhamnetin-3-glucoside) are reported on dry weight basis

[2]I-3-R-R was estimated using the calibration curve for I-3-R, corrected for molecular weight

[3]Total flavonoids are the sum of I-3-R-R, I-3-R and I-3-G

Table 18. Content of Dominant Flavonoid Compounds in Calendula Flowers Grown in the USA in 2000.

Sample ID		Harvest Date	Moisture (%)	I-3-R-R [1,2] (%)	I-3-R[1] (%)	I-3-G[1] (%)	Total Flavonoids[3] (%)
Madras, OR	R1, R2	6 July	11.59	0.74	0.57	0.02	1.33
"	R3, R4	"	10.09	0.76	0.61	0.02	1.39
"	R1, R2	17 August	9.46	0.68	0.79	0.03	1.50
"	R3, R4	"	9.33	0.76	0.76	0.03	1.55
"	R1, R2	12 September	11.41	0.52	0.85	0.04	1.41
"	R3, R4	"	11.59	0.52	0.83	0.04	1.39
Mean±S.D.				**0.66±0.11**	**0.74±0.12**	**0.03±0.01**	**1.43±0.08**
Davis, CA	R1, R2	17 July	9.27	0.54	0.35	0.02	0.91
"	R3, R4	"	9.66	0.54	0.40	0.01	0.95
"	R1, R2	21 August	9.38	0.36	0.31	0.05	0.72
"	R3, R4	"	9.57	0.35	0.28	0.05	0.68
"	R1, R2	11 September	9.23	0.30	0.43	0.06	0.79
"	R3, R4	"	9.05	0.29	0.40	0.06	0.75
Mean±S.D.				**0.40±0.11**	**0.36±0.06**	**0.04±0.02**	**0.80±0.11**

[1]Content of I-3-R-R (isorhamnetin-3-O-2-rhamnosyl-rutinoside), I-3-R (isorhamnetin-3-rutinoside) and I-3-G (isorhamnetin-3-glucoside) are reported on dry weight basis

[2]I-3-R-R was estimated using the calibration curve for I-3-R, corrected for molecular weight

[3]Total flavonoids are the sum of I-3-R-R, I-3-R and I-3-G

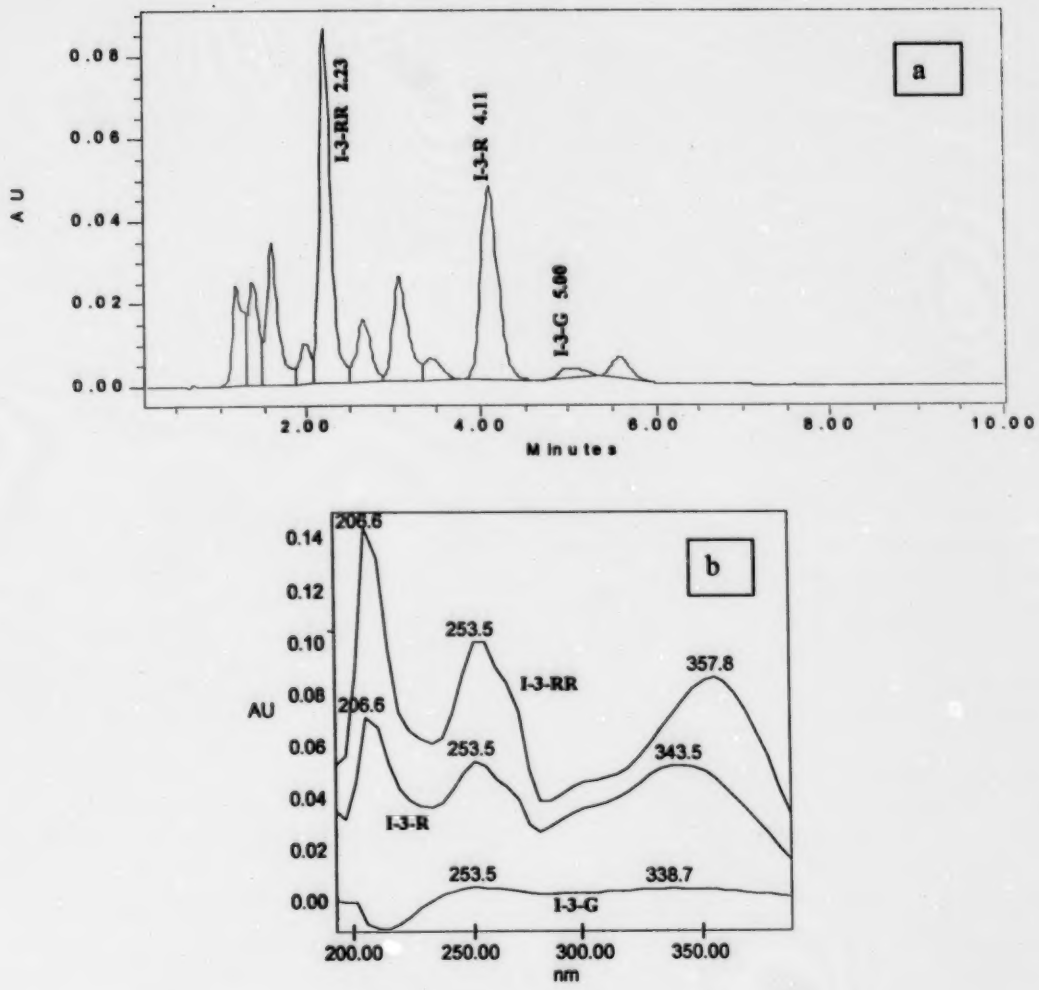


Figure 13. HPLC profile (a) and UV spectra (b) of flavonol glycosides in calendula flowers (I-3-RR = isorhamnetin-3-rhamnosylrutinoside; I-3-R = isorhamnetin-3-rutinoside; I-3-G = isorhamnetin-3-glucoside).

The content of three flavonol glycosides in calendula flowers collected in 1999 at 3 harvest dates in La Ronge, Outlook and Saskatoon is presented in Table 19. The total content ranged from 0.54% to 1.80%, and on average was the highest in La Ronge, followed by Saskatoon and Outlook. This year calendula flowers from Madras contained 1.25% flavonoids as compared to Saskatchewan average of 1.09%. The level of flavonoids was the highest in Madras samples in 2000 as well, 1.43% as compared to 1.03% Saskatchewan average. Samples from Davis contained the least amount of flavonoids (0.71% to 0.8%) in both years of study. As anticipated, the time of harvest had an obvious impact on the composition of medicinal constituents in calendula flowers. The content of flavonol glycosides showed a decreasing trend from July to September, which is in agreement with the results from our former studies. The flavonol glycosides content was significantly higher in 1999 than in a similar study in 1997 (ADF project #96000172).

Table 19. Content of Dominant Flavonoid Compounds in Calendula Flowers Grown at all five locations in 1999.

Sample ID	Harvest Date	I-3-R-R[1,2] (%)	I-3-R[1] (%)	I-3-G[1] (%)	Total Flavonoids[3] (%)
La Ronge					
"	7 July	0.90±0.13	0.88±0.12	0.02±0.00	1.80
"	18 Aug.	0.53±0.12	0.51±0.13	0.02±0.00	1.06
"	14 Sept.	0.51±0.04	0.49±0.04	0.03±0.00	1.03
Mean±S.D.					***1.30±0.44***
Outlook					
"	6 July	0.64±0.06	1.00±0.15	0.05±0.01	1.69
"	17 Aug.	0.29±0.05	0.24±0.05	0.01±0.00	0.54
"	1 Sept.	0.38±0.03	0.30±0.04	0.01±0.00	0.69
Mean±S.D.					***0.97±0.63***
Saskatoon					
"	5 July	0.50±0.05	0.75±0.02	0.02±0.01	1.27
"	16 Aug.	0.47±0.07	0.55±0.09	0.03±0.02	1.05
"	27 Aug.	0.41±0.05	0.30±0.07	0.01±0.00	0.72
Mean±S.D.					***1.01±0.28***
Madras					
"	5 July	0.78±0.04	0.84±0.09	0.02±0.01	1.64
"	16 Aug.	0.43±0.03	0.44±0.10	0.01±0.00	0.88
"	13 Sep.	0.48±0.06	0.72±0.14	0.02±0.01	1.22
Mean±S.D.					***1.25±0.38***
Davis					
"	13 July	0.49±0.04	0.37±0.03	0.01±0.00	0.87
"	11 Aug.	0.44±0.02	0.43±0.08	0.01±0.00	0.88
"	15 Sep	0.22±0.03	0.17±0.02	-	0.39
Mean±S.D.					***0.71±0.28***

[1]Content of I-3-R-R (isorhamnetin-3-O-2-rhamnosyl-rutinoside), I-3-R (isorhamnetin-3-rutinoside) and I-3-G (isorhamnetin-3-glucoside) are reported on dry weight basis

[2]I-3-R-R was estimated using the calibration curve for I-3-R, corrected for molecular weight

[3]Total flavonoids are the sum of I-3-R-R, I-3-R and I-3-G

The variation in the content of natural compounds in plant material is influenced by a number of factors. Therefore, observations of trends rather than comparison of single harvest data appear to be more reasonable approach. Calendula flowers grown in Davis contained consistently the lowest amount of flavonol glycosides, which may be a result of inadequate post-harvest treatment, possible latitudinal effect on phytomedicinal quality or some other factors. Overall, calendula flowers grown in Madras were of best quality, particularly in 2000. While Saskatchewan may not have a competitive advantage over Madras in calendula production, it has certainly been proven that good quality calendula may be grown as far north as La Ronge, providing recommended harvest and post-harvest handling procedures are followed.

14.0 *Silybum mariaum* (Milk Thistle)

Milk thistle seed has been widely used for the treatment of various liver conditions. It is commonly available in form of a standardized extract containing 70% of several flavonolignans known as silymarin complex, or simply silymarin.

Objective

1. to determine silymarin content in milk thistle seed by HPLC.

Materials and Methods

Plant Material

Milk thistle seed from 4 different sources [Bulgarian, specific site unknown; Richters (Ontario); Horizon Herbs (Oregon); Johny's Selected Seeds (Maine)] was grown at the horticulture research plot in Saskatoon in 2001. Since plants were transplanted on June 5 (Bulgarian, Horizon Herbs and Johny's) and June 25 (Richters), multiple harvests were conducted between September 5 and October 12 to allow for seed from all sources to reach a same stage of maturity. Seed heads were collected manually and dried at 35°C. Then, the seed was trashed and cleaned by size by passing it through a set of appropriate sieves. Final seed cleaning based on gravity was conducted on a piece of equipment kindly provided by the Department of Agriculture and Bioresource Engineering, University of Saskatchewan. Seed was made to vibrate in a fluidized type bed by air under pressure. By careful control of air flow, light matter such as chaff, immature and aborted seed, and other light foreign matter were overflown to a separate compartment and discarded. The remaining seed was collected and stored at dry place at 10°C until analyzed. It consisted of 28 samples: 12 samples from combined harvests of September 5, 12 and 19, and 16 samples of October 4 and October 12 harvests.

Sample Preparation

Clean dry milk thistle seed was ground to a fine powder using Thomas-Wiley Laboratory Mill and passed through a 24 mesh sieve (particle size 710 micrometers). It was analyzed for the contents of fixed oil and flavonolignans known as silymarin complex by HPLC.

Seed Extraction for Fixed Oil and Silymarin

One gram of powdered seed was extracted in a Soxhlet extractor with petroleum ether for 4 hour to separate fixed oil. The oil was recovered by evaporating the solvent to dryness under vacuum at 40 °C and weighing the oily residue. The seed sample was subsequently extracted with 100 ml of methanol in a Soxhlet extractor for 4 hr to extract silymarin complex. Methanol was evaporated to dryness under vacuum at 40 °C and the residue was re-dissolved in 25 ml of methanol. One ml aliquot of methanol extract was filtered through a 0.45 µm syringe filter and subjected to HPLC analysis.

Liquid Chromatography

The HPLC analysis of silymarin complex, which includes silybinin A, silybinin B, dehydrosilybinin, silychristine, silydianine and taxifolin, was conducted on a Waters system (Milford, MA, USA) comprised of a Waters 600 system controller, a Waters 996 diode array detector, and a Waters 717 Plus auto injector using Novapak C-18 column (150 mm *x* 3.9 mm, 5µm) at 25 °C. The mobile phase consisted of 45 % methanol and 55 % aqueous buffer, pH 2.2; the injection volume was 5 µL and the isocratic flow rate was 1 mL/min. Silymarin constituents were detected at 290 nm. Silymarin content was determined as the sum of the following flavonolignan compounds: silibinin [A,B, dihydro (DH-)], silychristine, silydianine and taxifolin.

Results

The HPLC profile of mature milk thistle seed extract is presented in Fig. 14. Peak assignment was done on the basis of findings from previous studies conducted by the HRP. The effect of seed source and harvest date on the percentage fixed oil, silymarin, taxifolin, silychristine, silydianine, silybinin A, silybinin B, and DH-silybinin content of milk thistle seed is presented in Table 20 and 21. In general, silymarin content was lower in the early harvest (0.83 – 1.82%) in comparison to 2.2-2.4% % in the late harvest of October 4 and 12. This year in this particular trial seed did not reach 3% silymarin level which is considered desirable for seed of high quality. Previous studies by Herb Research Program and collaborators suggested that milk thistle quality is greatly impacted by seed maturity. Low silymatin levels, below 1.8%, in seed harvested by the middle of September likely reflects seed immaturity.

Milk thistle seed with the highest content of silymarin (2.21-2.37%) was harvested on October 4 and October 12, which suggests that 115-120 days from transplanting to harvest were required for this crop. While this long growing season presents an obstacle for a harsh prairie climate, a hard frost (below-5°C) did not seem to affect silymarin content. Data presented in Table 21 provides first indication that milk thistle can be harvested after a hard frost without compromising crop quality. This aspect of flexible harvest date is a critical factor in the success of milk thistle as a medicinal crop for Saskatchewan.

Fixed oil content was the highest in seed harvested on October 4 and ranged from 22.8 to 26.1% between seed sources. Bulgarian and Richters seed contained highest amount of both fixed oil and silymarin. Silychristine and silybinin A and B were the main silymarin constituents as expected in both Bulgarian and Richters seed.

Overall, seed from Bulgaria performed the best in a 2001 trial in Saskatoon, followed by Richters seed. Seed from Horizon Herbs and Jonny's performed poorly and will be excluded from future testing. In conclusion, milk thistle has some potential as a medicinal seed crop for Saskatchewan, but further research into optimizing seeding date, improving uniformity of seed maturation, and examining the effect of various seed sources on seed medicinal quality remains to be done in order to optimize and improve consistency of silymarin content.

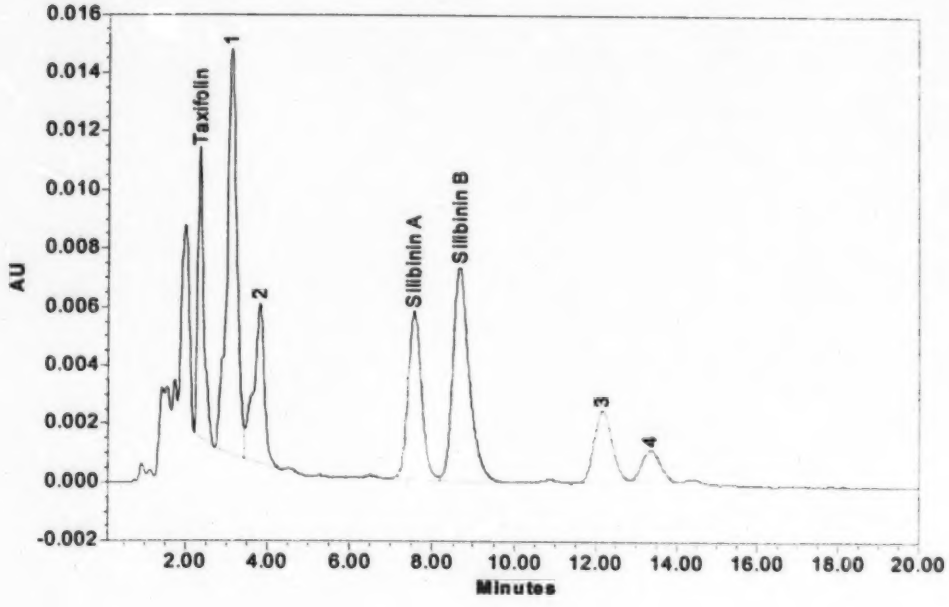


Figure 14. HPLC profile of mature milk thistle seed extract [peaks 1, 2, 3, and 4 correspond to silychristine, silydianine, dihydroxysilybinin, and an unknown compound, respectively).

Table 20. Effect of Harvest Date and Seed Source on the Content of Silymarin and Fixed Oil in Milk Thistle Seed (Saskatoon, 2001).

Sample Source/ Lab ID	Rep #	Fixed Oil (%)	Taxifolin[1] (%)	Silychristine[1] (%)	Silydianine[1] (%)	Silybinin A[1] (%)	Silybinin B[1] (%)	DH-Silybinin[1] (%)	Silymarin[1,2] (%)
Pooled Harvest: September 5+12+19									
Bulgaria/	1	24.19	0.14	0.48	0.22	0.30	0.43	0.19	1.75
"	2	27.16	0.11	0.35	0.19	0.22	0.28	0.13	1.28
"	3	25.27	0.24	0.68	0.16	0.49	0.69	0.20	2.46
"	4	23.06	0.14	0.49	0.19	0.33	0.47	0.19	1.80
	Mean±SD	**24.92±1.74**	**0.16±0.06**	**0.50±0.13**	**0.19±0.02**	**0.33±0.12**	**0.47±0.17**	**0.18±0.03**	**1.82±0.48**
Johnny's Selected Seeds/	1	18.09	0.11	0.14	0.22	0.08	0.11	0.15	0.80
"	2	19.85	0.09	0.13	0.19	0.08	0.11	0.15	0.76
"	3	20.28	0.10	0.17	0.28	0.09	0.11	0.17	0.92
"	4	23.61	0.11	0.15	0.24	0.08	0.11	0.16	0.85
	Mean±SD	**20.46±2.31**	**0.10±0.01**	**0.15±0.01**	**0.23±0.04**	**0.09±0.00**	**0.11±0.00**	**0.16±0.01**	**0.83±0.07**
Horizon Herbs/	1	19.22	0.12	0.17	0.26	0.09	0.12	0.19	0.96
"	2	20.21	0.14	0.29	0.36	0.12	0.17	0.20	1.27
"	3	21.56	0.10	0.13	0.31	0.09	0.11	0.16	0.90
"	4	22.35	0.14	0.22	0.39	0.09	0.12	0.19	1.15
	Mean±SD	**20.84±1.39**	**0.12±0.02**	**0.20±0.07**	**0.33±0.06**	**0.10±0.02**	**0.13±0.03**	**0.18±0.02**	**1.07±0.17**

[1]Contents of fixed oil, silibinin [A,B, dihydro (DH-)], silychristine, silydianine, taxifolin and silymarin in the seed are reported on as is basis

[2]Silymarin content is reported as the sum of silibinin [A,B, dihydro (DH-)], silychristine, silydianine and taxifolin

Table 21. Effect of Harvest Date and Seed Source on the Silymarin and Fixed Oil Content in Milk Thistle Seed Grown in Saskatoon in 2001.

Sample Source/ Lab ID	Rep #	Fixed Oil (%)	Taxifolin[1] (%)	Silychristine[1] (%)	Silydianine[1] (%)	Silybinin A[1] (%)	Silybinin B[1] (%)	DH-Silybinin[1] (%)	Silymarin[1,2] (%)
Harvest: October 4									
Bulgaria/	1	24.79	0.24	0.68	0.16	0.48	0.66	0.22	2.43
"	2	26.25	0.17	0.58	0.14	0.39	0.54	0.18	2.00
	Mean±SD	**25.52±1.03**	**0.20±0.05**	**0.63±0.07**	**0.15±0.02**	**0.44±0.06**	**0.60±0.09**	**0.20±0.02**	**2.21±0.30**
Johnny's Selected Seeds	1	22.40	0.12	0.22	0.26	0.09	0.11	0.16	0.96
"	2	23.31	0.13	0.18	0.25	0.09	0.12	0.19	0.96
	Mean±SD	**22.86±0.64**	**0.12±0.01**	**0.20±0.03**	**0.26±0.00**	**0.09±0.00**	**0.12±0.01**	**0.18±0.02**	**0.96±0.01**
Horizon Herbs/	1	23.70	0.12	0.23	0.30	0.09	0.12	0.17	1.03
"	2	22.61	0.12	0.23	0.31	0.09	0.12	0.18	1.05
	Mean±SD	**23.16±0.77**	**0.12±0.00**	**0.23±0.00**	**0.30±0.01**	**0.09±0.00**	**0.12±0.00**	**0.18±0.01**	**1.04±0.02**
Richters/	1	25.47	0.12	0.64	0.13	0.52	0.74	0.20	2.34
"	2	26.49	0.14	0.72	0.12	0.59	0.80	0.22	2.59
"	3	26.62	0.16	0.57	0.12	0.48	0.68	0.19	2.19
	Mean±SD	**26.19±0.63**	**0.14±0.02**	**0.65±0.08**	**0.12±0.00**	**0.53±0.06**	**0.74±0.06**	**0.20±0.01**	**2.37±0.203**
Harvest: October 12									
Bulgaria/	1	24.77	0.23	0.64	0.19	0.42	0.58	0.23	2.30
"	2	24.96	0.24	0.66	0.19	0.44	0.62	0.23	2.37
	Mean±SD	**24.87±0.13**	**0.24±0.01**	**0.65±0.01**	**0.19±0.00**	**0.43±0.01**	**0.60±0.03**	**0.23±0.01**	**2.33±0.05**
Johnny's Selected Seeds	1	25.17	0.10	0.20	0.20	0.11	0.14	0.15	0.90
"	2	23.92	0.11	0.19	0.25	0.09	0.11	0.18	0.93
	Mean±SD	**24.55±0.88**	**0.11±0.01**	**0.19±0.01**	**0.22±0.04**	**0.10±0.02**	**0.13±0.02**	**0.16±0.02**	**0.91±0.02**
Horizon Herbs/	1	24.04	0.11	0.19	0.22	0.10	0.11	0.16	0.89
"	2	22.89	0.12	0.22	0.23	0.10	0.13	0.17	0.96
	Mean±SD	**23.47±0.81**	**0.11±0.01**	**0.20±0.02**	**0.23±0.01**	**0.10±0.00**	**0.12±0.01**	**0.17±0.01**	**0.92±0.05**
Richters/	1	22.83	0.16	0.69	0.12	0.548	0.77	0.21	2.50

[1]Contents of fixed oil, silibinin [A,B, dihydro (DH-)], silychristine, silydianine, taxifolin and silymarin in the seed are reported on as is basis

[2]Silymarin content is reported as the sum of silibinin [A,B, dihydro (DH-)], silychristine, silydianine and taxifolin

15.0 Tanacetum parthenium (Feverfew)

Feverfew is best known for its use in the treatment of migraine headaches. It is believed that parthenolide, a sesquiterpene lactone type compound, is partially responsible for this activity of feverfew. A dose of 125-250 mg daily of dried feverfew leaf preparation containing a minimum of 0.2% parthenolide is allowed by Health Canada in over the counter medication (e.g., Tanacet).

Objective

1. to determine parthenolide content in aerial parts of 3 different feverfew seed sources by HPLC.

Materials and Methods

Plant Material

Feverfew from three seed sources, Richters (Ontario), Horizon Herbs (Oregon), and Johnny's Selected Seeds (Maine) were grown at the horticulture research plot in Saskatoon. Leaves and flowers of feverfew were collected separately and stored in dry, dark place at 10°C until analyzed.

Sample Preparation

Prior to analysis plant material was ground to a fine powder with particle size below 1000 µm using Thomas-Wiley Laboratory Mill. A total of 0.5 g of powdered plant material was extracted with methanol by shaking for 10 min at 60°C, extract was cooled and filtered through Whatman 202 filter paper and the final volume adjusted to 25 mL.

Liquid Chromatography

Samples were analyzed by the HPLC (Waters system with the Millennium 32 Chromatography Manager software) on a Novapak C18 column (4µm, 150 x 3.9 mm). The mobile phase was acetonitrile:water (40:60) pumped at a flow rate of 1 mL/min. The injection volume was 20 µL. The elution program consisted of a 20 min isocratic elution followed by a 2 min acetonitrile wash. With the 15 min next injection delay, total time per sample was 35 min. Parthenolide was detected by PDA at 210 nm. Seven-point calibration curve was generated using parthenolide (Sigma, Canada) in methanol in the range of 500–2500 ng.

Results

The floral portion of plants from all three sources consistently contained the highest level of parthenolide (Table 22). Parthenolide content was the lowest in pre-flowering leaves followed by leaves, top half of the plant and flowers, and was in the range from 0.06% to 1.16 %. Flowers of plants grown from both Richters and Horizon Herbs seed contained equally high amount of parthenolide, 1.10% to 1.16%, which suggests that seed from these sources should be used in future studies. Plants from

Johnny's Selected Seeds (Maine, USA) had significantly lower parthenolide content than plants from other seed sources. Results from Table 22 indicate the height of the cut of feverfew plants has a great impact on quality, which is in agreement with the previous studies of HRP. It should be noted that in the top half of plants parthenolide content was more than 50% lower than in flowering tops, which is in agreement with other reports. Feverfew of good quality can be grown as an annual plant in Saskatchewan, but Saskatchewan does not appear to have any competitive advantage over other locations in growing this medicinal plant.

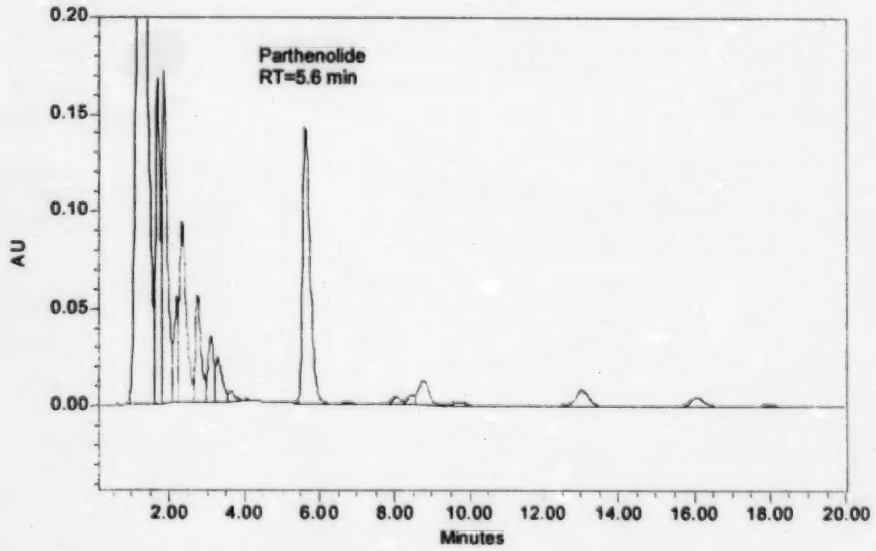


Figure 15. HPLC profile of the extract of feverfew flowers.

Table 22. Parthenolide Content in Feverfew Samples Grown in Saskatoon in 2001.

Seed Source	Plant Part Harvested		Harvest Date	Parthenolide (%)	Parthenolide Mean±SD (%)
Richters					
"	Top Half	#1	Aug 10	0.66	
"	"	#2	"	0.53	0.61±0.07
"	"	#3	"	0.64	
"	Leaves	#1	"	0.31	
"	"	#2	"	0.29	0.30±0.01
"	"	#3	"	0.29	
"	Flowers	#1	"	1.21	
"	"	#2	"	1.20	1.16±0.08
"	"	#3	"	1.07	
Johnny's					
	Pre-flowering leaves	#1	Aug 13	0.08	
"	"	#2	"	0.03	0.06±0.02
"	"	#3	"	0.06	
"	Top half	#1	Sep 5	0.22	
"	"	#2	"	0.12	0.13±0.09
"	"	#3	"	0.05	
"	Leaves	#1	"	0.05	
"	"	#2	"	0.12	0.09±0.04
"	"	#3	"	0.11	
"	Flowers	#1	"	0.29	
"	"	#2	"	0.69	0.64±0.32
"	"	#3	"	0.94	
Horizon					
	Pre-flowering leaves	#1	Aug 13	0.15	
"	"	#2	"	0.16	0.15±0.00
"	"	#3	"	0.16	
"	Top half	#1	Sep 7	0.43	
"	"	#2	"	0.38	0.42±0.03
"	"	#3	"	0.44	
"	Leaves	#1	"	0.19	
"	"	#2	"	0.19	0.20±0.01
"	"	#3	"	0.22	
"	Flowers	#1	"	1.06	
"	"	#2	"	1.15	1.11±0.04
"	"	#3	"	1.12	

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16.0 Personnel

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Donna Dunlop, Research Assistant (2 months)
Dr. Anna Szmigielski, Post-doctoral Research Fellow (3 months)
George Katselis, Graduate Student (1 month)
Dr. Branka Barl, Professional Research Associate (5% of 36 months)

17.0 Equipment

None

18.0 Project Developed Materials

Segments of the data generated on this project were presented at the annual meetings of the SHSA and other speaking engagements.

19.0 Project Photos

A collection of slides and photographs of plants from Saskatchewan and Oregon sites has been prepared.

20.0 SDAF Acknowledgment

Same as (e)